A COMPUTED TOMOGRAPHIC AND PATHOLOGICAL STUDY OF EQUINE CHEEK TEETH INFUNDIBULAR CARIES

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DECLARATION

I declare that the contents of this thesis are my own work and composition and that the work has not been submitted for any other degree or to any other university than the University of Edinburgh.

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CONTENTS

	Page
Title Page	1
Declaration	2
Acknowledgements	3
Contents	5
Abstract	9
1. Introduction and Literature Review	12
1.1 Equine dental evolution	12
1.2 Embryology and development of teeth	13
1.3 Dental physiology and mastication	15
1.4 Normal cheek tooth anatomy	16
1.4.1 Enamel	17
1.4.2 Dentine	18
1.4.3 Cementum	19
1.4.4 Dental pulp	19
1.4.5 Periodontal tissues	20
1.5 Infundibular cemental defects	21
1.5.1 Infundibular caries	21
1.5.2 Other cemental abnormalities	23
1.6 Sequelae associated with infundibular caries	25
1.6.1 Periapical infection	25
1.6.2 Cheek tooth fracture	26
1.6.3 Extension of periapical infection	29
1.6.4 Cheek tooth diagnostic imaging	30
1.7 Aims of this study	31
2. Materials and Methods	32
2.1 Sample collection	32
2.2 Occlusal surface assessment	33

2.3 Computed tomographic imaging 38

2.4 Pilot study of sectioning and histology of cheek teeth 40

2.5 MicroCT and histology 43

2.6 Statistical techniques 44

3. Results 45

3.1 Occlusal surface assessment 45

3.1.1 Caries prevalence 45

Relationship between occlusal infundibular caries and Triadan position 46

Relationship between occlusal infundibular caries and age group 49

3.2 Occlusal infundibular caries grade 50

3.2.1 Prevalence of occlusal surface caries grade 50

Relationship between grade of occlusal infundibular caries and age group 53

Relationship between grade of occlusal infundibular caries and Triadan position 54

3.3 Computed tomographic findings 55

3.3.1 Infundibular depth and crown length measurements 55

Relationship between ID:CL ratio and age group 57

Relationship between ID:CL ratio and Triadan position 57

Relationship between ID:CL ratio in infundibulae affected by and unaffected by occlusal caries 58

Relationship between ID:CL ratio and grade of occlusal infundibular caries 59

3.3.2 Computed tomographic examination of cheek teeth 60

Relationship between subocclusal infundibular lesions and Triadan position 61

Relationship between subocclusal infundibular lesions and age group 61

Relationship between subocclusal infundibular lesions and ID:CL ratio 62

3.3.3 Morphological characteristics of infundibular lesions on computed tomography 62

Relationship between type of subocclusal lesion and infundibular position (rostral or caudal) 65
Relationship between type of subocclusal infundibular lesion and Triadan position 66
Relationship between type of subocclusal infundibular lesion and age group 67
Relationship between occlusal and subocclusal infundibular lesions 68
3.3.4 Central linear cemental defects 70
Relationship between central linear cemental defects and occlusal infundibular caries 70
Relationship between central linear defects and infundibular position (rostral and caudal) 71
Relationship between central linear cemental defects and Triadan position 71
Relationship between central linear cemental defects and age group 72
3.3.5 Apical cemental hypoplasia 72
Relationship between apical cemental hypoplasia and occlusal infundibular caries 74
Relationship between apical cemental hypoplasia and Triadan position 75
Relationship between apical cemental hypoplasia and infundibular position (rostral or caudal) 75
Relationship between apical cemental hypoplasia and age group 76
3.3.6 Combined apical infundibular lesions 76
3.3.7 Occult subocclusal infundibular lesions in teeth without visible occlusal lesions 77
Relationship between occult subocclusal infundibular lesions and Triadan position 77
Relationship between occult subocclusal infundibular lesions and age group 78

3.4 MicroCT and histopathological findings 79
3.4.1 Density of infundibular contents in Hounsfield Units 89
4. Discussion 93
4.1 Sample selection and assessment 93

4.2 Occlusal surface: infundibular caries 95
4.3 Computed tomography 98
4.4 MicroCT and histology 102
5. Conclusions 110
6. References 112

Abstract

Several studies have found equine maxillary cheek teeth infundibular disorders to affect most adult horses. Even infundibulae that appear grossly normal on the occlusal surface may contain areas of caries, defective cementum, or complete absence of cementum subocclusally. These defects can range from possibly insignificant small developmental cemental defects deep in the infundibulum to advanced coalescing infundibular caries leading to dental fracture.

The aim of this study was to characterize maxillary cheek teeth infundibular disorders by gross, computed tomographic, and histopathological examinations of affected and control teeth. One hundred maxillary cheek teeth that contained 200 infundibulae were extracted post-mortem from 30 horses, including 82 teeth with and 18 without occlusal surface infundibular caries. The occlusal surface infundibular caries was graded using the modified Honma technique, which found 61.8% of the 82 affected teeth to be affected by Grade 1 caries, 27.9% with grade 2 and 10.3% with grade 3 caries. The rostral infundibulae were more commonly affected by occlusal surface lesions (72% affected) as compared to the caudal infundibulae (64% affected) and also had higher grades of occlusal caries. The Triadan 09 position was greatly overrepresented in the diseased teeth, comprising 50% (41/82) of teeth with visible occlusal caries. There was a significant association between the grade of occlusal infundibular caries present and the Triadan positions of the teeth.

All teeth were then imaged by computed tomography (CT) to determine crown and infundibular lengths, and to detect the presence and appearance of any subocclusal infundibular cemental abnormalities. Extensive statistical analysis of dental crown and infundibular lengths were performed, showing that relatively shorter infundibulae are more likely to be diseased. However, this finding can be explained by the predominance of Triadan 09 and older teeth in the diseased group, both of which have lower infundibular depth:crown length ratios. Computed tomography showed 182/200 (91%) infundibulae to have infundibular lesions deep to the occlusal surface. No statistically significant association was found between the presence of subocclusal infundibular defects and age of affected horses, grade of caries, or the presence of visible occlusal infundibular lesions. Teeth affected by occlusal caries were 1.3 times more likely to have subocclusal lesions than teeth without occlusal caries. This study also proposes a relationship between the presence of developmental infundibular cemental lesions and later caries formation.

Eight maxillary cheek teeth were then imaged using micro-computed tomography (microCT) which provided much finer details of infundibular lesions. MicroCT images were also used to guide the sites of sectioning of teeth for histopathological analysis, which found that the appearance of infundibular cementum was much more variable than its appearance on standard CT or microCT. The cementum of many infundibulae showed extensive sites of former vasculature that were not filled with cementum. Other areas contained moth-eaten cement which often contained plant material and cellular debris, independent of the appearance of the occlusal surface of the infundibulum. No histological evidence of previous vasculature or of haemoglobin breakdown products were found in infundibular cementum.

Major findings and interpretations of this study allow us to conclude:

- Many infundibulae are affected by occult subocclusal cemental lesions. These lesions appear to have no association with the presence or absence of apparent occlusal surface infundibular lesions, and also are not more significantly apparent in particular Triandan positions or in horses of a particular age. As the subocclusal cemental lesions appear to be unrelated to the presence of occlusal surface lesions, the relationship between infundibular cemental lesions (both occlusal and subocclusal) and the development of clinical sequelae remains poorly understood.
- Even in those infundibulae with no apparent occlusal surface infundibular caries, the subocclusal region often contained hypoplastic cementum and, often, impacted feed material at the most apical aspect of the infundibulum on histological examination. Therefore, there must be a communication between the apical portion of the infundibulum and the occlusal surface for this to occur, even in those that appear unaffected by a vascular channel defect or infundibular caries.
- The strict definition of infundibular caries as opposed to normal cementum on the occlusal surface of infundibulae is inadequate to describe the great variation in the appearance and composition of infundibular cementum. Further classification of the subocclusal cemental defects and expectations of normal subocclusal cementum may also be included for a more complete understanding. There should be a standardisation of terminology used to describe normal and defective infundibular regions both on the occlusal surface and subocclusally.

- It is likely that subocclusal cemental lesions, particularly areas of apical cemental hypoplasia, become become impacted with feed material even before they are exposed on the occlusal surface, which may allow severe (Grade 2 or 3) infundibular caries to form subocclusally long before that portion of the tooth is exposed.
- Analysis of the Hounsfield Units (measurement of density of tissues on CT scan) and their relationship to the histological appearance of the same tissues may allow us to determine if an infundibulum contains normal or hypoplastic cementum, or impacted feed material without more invasive investigatory methods.

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1. Introduction and Literature Review

1.1 Equine dental evolution

The earliest known predecessors of the modern equid, known as eohippus or *Hyracotherium*, inhabited North America between 50 and 55 million years ago (Bennett 1992). Its diet consisted mostly of soft plants containing simple carbohydrates and had low silica content, and therefore their dentition varied greatly from that of their modern descendants *(Equus caballus)*. These earliest equine teeth were brachydont, or low-crowned, with the clinical crowns being composed primarily of dentine fully covered by enamel. The teeth of *Hyracotherium* had a predetermined lifespan of approximately 5 years as opposed to the 20-25 year predicted lifespan of the teeth in the modern horse (MacFadden 1999).

Climatic changes about 50 million years ago in North America led to a gradual change in vegetation to coarser, cellulose-containing grasses that, by necessity, led to a transition in Hyracotherium's diet to more fibrous materials, and alteration from browsing to grazing methods of ingestion. All mammals are unable to digest cellulose, but the hindgut of the early equid enlarged greatly to create a favorable environment in which cellulase-containing microbes could thrive. These micro-organisms were able to digest cellulose and release some of its nutrients to be used by the horse. This low-energy diet necessitated horses ingest and masticate large volumes of tough, silicate-containing food for up to 18 hours a day (Bennett 1992).

Consequent to this dietary change, equine dentition had to undergo significant evolution as well. Over the first 20 million years of equine evolution, the second to fourth premolars became molar-like in appearance, with no apparent differentiation between modern equine premolars and molars remaining today. Consequently, the modern horse has 6 anatomically identical premolars and molars in each arcade termed collectively as 'cheek teeth'. The first premolar, or wolf tooth, however, became vestigial in nature and is typically a small peg-shaped tooth up to 2 cm in length. They are typically found in the maxillary arcade in 15 – 21% of adult horses (Colyer 1990).

The development of hypsodont (high-crowned) and continuously erupting, teeth also coincided with increased grazing behavior in the horse. The jaws also became deeper to allow the high-crowned teeth to fit appropriately (Budiansky 1997). As the hypsodont dentition developed, enamel and dentine infoldings also began to form to increase the length of protruding enamel folds on the occlusal surface of the cheek teeth. The high-crowned teeth of modern horses also contain an increased amount of cementum in comparison to their predecessors. Because of the prolonged eruption of equine teeth, cementum appeared as a component of the equine clinical crown where it greatly contributes to the crown size (Mitchell 2003) as opposed to brachydont teeth in which it only appears subgingivally to anchor the periodontal ligaments. Additionally, the softer cementum and dentine serve to buffer the more brittle adjacent enamel against cracking or fracturing (Bennett 1992). Peripheral cementum is present over the entire non-occlusal external surface of the equine tooth, serving subgingivally as part of the periodontal apparatus (Butler 1991), while infundibular cementum fills the enamel infundibulae in the incisors and maxillary cheek teeth.

1.2 Embryology and development of teeth

Dentogenesis begins with formation of the dental lamina, a ridge of epithelial tissue that folds into the mesenchyme of the foetal oral cavity (Ten Cate et al 2003). The dental lamina becomes hollow and later underlying mesenchymal cells migrate into its base. These two tissues produce tooth buds which eventually develop into teeth, along with a dental sac which nourishes and protects the enamel-producing organ within each developing tooth. The tooth bud progresses to the “cap stage” with the formation of the enamel organ. The hollow portion of the enamel organ becomes filled with dental papilla which will later become pulp and dentine. The enamel organ, dental papilla, and dental sac together form the tooth germ. As the enamel organ matures, the developing tooth is termed to be at the “bell stage” of development. The odontoblasts lay down dentine along the basal membrane which induces adjacent epithelial cells within the enamel organ to differentiate into ameloblasts and produce the enamel with its infoldings when stimulated. The infundibulae of maxillary cheek teeth and incisors also begin as enamel invaginations at the convex aspect of this bell (Dixon and du Toit 2010).

The process of cytodifferentiation continues with the differentiation of the uppermost papillary cells into odontoblasts which produce dentine. As dentine production continues in an apical direction, the contact of dentine with the dental sac induces the formation of cementoblasts and subsequent cementum production. The production of dentine and enamel both begin with the deposition of specific dental mucopolysaccharides to create a matrix framework for later mineralization. The mineralization of teeth begins occlusally at each individual cusp tip, and then proceeds apically, towards the amelodentinal junction. The dental sac provides a source of nourishment for the developing infundibular enamel and cementum until the time of eruption and then wear of the tooth. Until mineralization is complete at the apex of the tooth, blood vessels from the mesenchyme can nourish the enamel as well (Berkovitz and Moxham 1981).

Once enamel deposition is complete, the ameloblasts that laid down enamel both peripherally and within the infundibulae die, and thus there can never be enamel repair. Within the infundibulae, cementum deposition begins along the central vascular channel. Vasculature from the dental sac enters through the central aspect of the occlusal surface of the infundibulum, which intersects with vessels that enter through the caudal aspect of the caudal infundibulum and more apically to the occlusal surface, through the rostral aspect of the rostral cheek tooth infundibulum and caudal aspect of the caudal infundibulum (Suske et al 2016a). This creates one vessel coursing through the central portion of each infundibulum that supports cemental deposition within the infundibulum. Once the tooth erupts, the occlusal blood supply is destroyed as the dental sac is worn away. However, infundibular cemental deposition can continue to be nourished by the additional lateral vasculature. Variable amounts of viable vasculature have been found apically in some dentition for many years following eruption (Fitzgibbon et al 2010). Recently, Suske et al (2016a) clarified that additional lateral infundibular vasculature is only present in the occlusal portion of the infundibulum, as opposed to previous studies which found that it entered through the apex of the developing infundibulum (Fitzgibbon et al 2010; Dixon and du Toit 2010). The deposition of cementum within the infundibulae is then limited by the amount of viable vasculature which remains (Kilic et al 1997b; Mitchell 2003). Gingival vasculature supports further deposition of peripheral cementum around the clinical crown, particularly after the tooth erupts from the rigid confines of the alveolus, and for root formation for its entire lifespan (Mitchell 2003).

The fully formed equine cheek tooth usually contains at least 5 vascular pulp horns (with 6 in all Triadan 06 cheek teeth, 6 in mandibular Triadan 11 cheek teeth, and 7 in maxillary cheek teeth 11), all of which communicate via a common apical pulp chamber for the first few years after eruption but later develop variable patterns of pulp horn connections (Dacre et al 2008b; Kopke et al 2012). Hypsodont teeth constantly lay down secondary dentine at the occlusal and peripheral aspects of the pulp horns for up to 30 years. Therefore, equine pulp is required to remain metabolically active and maintain an excellent blood supply through larger vascular foramina than are present in brachydont teeth. The entire equine tooth is covered by a thin layer of cementum at eruption. The pulp horns are also covered by a layer of primary dentine, only at eruption, which wears during occlusion and is replaced by secondary dentine. Each pulp chamber is also surrounded by primary dentine which is surrounded by peripheral enamel. As noted, maxillary cheek teeth also contain 2 infundibulae lined by enamel and which should be filled with cementum. The folded layers of enamel surrounded by peripheral cementum covering the entire surface of the tooth then complete the tooth structure. When the tooth comes into attrition, or normal dental wear, secondary dentine begins to be laid down over (subocclusally) the pulp horns to protect them from exposure to the oral cavity environment and its microorganisms, as the pulps must remain viable throughout the life of the tooth. Pulpar exposure on the occlusal surface is often a consequence of apical infection and death of the tooth as the periodontal tissues are an independent system and continue to function in dental eruption even if the tooth's endodontic system has died. Secondary dentine is no longer produced to protect and cover the pulp, and as the secondary dentine becomes worn, the pulp horns become exposed to the surrounding environment (Dixon et al 2010).

1.3 Dental physiology and mastication

Infundibulae are present in both maxillary cheek teeth and incisors in the modern horse. They are comprised of an infolded enamel lined cup opening onto the occlusal surface that theoretically should be filled with cementum. The incisors contain 1 infundibulum each, while there are 2 infundibulae in each maxillary cheek tooth. Mandibular cheek teeth do not contain infundibulae, but they do contain a greater degree of peripheral enamel infoldings than the maxillary teeth (du Toit et al 2008). Maxillary cheek teeth infundibulae likely evolved to give a greater length of enamel folds on the occlusal surface to compensate for the more extensive enamel infolding present in the mandibular cheek teeth. The presence of enamel within the central area of the maxillary cheek teeth imparts further strength to the tooth structure including the masticatory surface. The combined structure of three different calcified dental substances (cement, enamel and dentine) along with the irregular pattern of enamel folds creates a self-sharpening mechanism on the occlusal surface. Different components of the tooth wear at varying rates, but overall, the contemporary equine tooth erupts and wears at a rate of about 2-3 mm per year (Dyce et al 2002). Enamel wears most slowly, dentine wears most quickly, and the rate of cemental wear cementum lies in between. Excessive wear in portions of or in an entire tooth occlusal surface is attributed to reduced enamel content or delayed eruption of that tooth respectively, which then leads to the development of overgrowth in the opposing tooth (Dixon et al 2000a).

The rate of attrition, or normal wear of the teeth, is determined by tooth morphology, diet, and the amount of time horses spend masticating. In the wild, horses may spend 16-18 hours per day grazing and masticating forage, while domesticated horses that are fed concentrates spend much less time eating and masticating. Eating concentrates encourages horses to have a much greater vertical crushing stroke when masticating along with less range in their medially directed horizontal power stroke than when masticating forage (Bonin et al 2007). The level and direction of occlusal forces on the teeth, and therefore patterns of attrition, change significantly depending on the diet of the individual horse. The interaction between all calcified tissues also affects the pattern of wear. The presence of slow-wearing enamel infoldings along with a vertical chewing motion due to eating of concentrate feeds can lead to development of sharp enamel points or overgrowths as the remaining components of the tooth wear more quickly (Leue 1941). The normal protruding enamel cusps that form as adjacent dentine and cementum wear more quickly are known as transverse ridges, and also function to increase the occlusal surface area but may become exaggerated due to abnormal patterns of wear (Dixon and Dacre 2005).

1.4 Normal cheek tooth anatomy

Equine dentition is classified as diphydont, meaning that horses have deciduous and permanent sets of teeth, and also as anelodont, meaning their teeth have limited growth time. Hypsondonty is a sub-division of anelodont teeth, meaning high-crowned. Equine teeth have prolonged eruption, usually over 20-25 years, with the crown becoming shorter over time. Hypsodont crowns are divided into the clinical, or erupted, crown and the reserve crown that is embedded in alveoli. Equine teeth do not have true roots (enamel free areas) at the time of eruption, and these cemental extensions begin to develop within 1 to 2 years of eruption. As cementum is deposited both at the roots and peripherally on the reserve crown, it helps to anchor the tooth within the alveolus through its attachment of the periodontal ligament (Colyer 1990). In the maxillary cheek teeth and incisors, cementum is also deposited within each infundibulum, and as noted, its occlusal deposition may eventually impede the blood supply to the deeper portion of the infundibulum. However, the alveolar blood supply to the pulp and periodontal membranes remains throughout the life of the tooth. The metabolically active hypsodont tooth can allow areas of pulpar exposure or areas of noxious stimuli to the pulp, such as adjacent caries, to be sealed with tertiary dentine in order to limit further pathological changes (Dacre et al 2008c).

Adult horses have between 36-44 permanent teeth. The typical dental formula includes 12 incisors (6/6), up to 4 canines (2/2), up to 4 first premolars (wolf teeth), and 24 cheek teeth. Canines only erupt in 27.2% of female horses (Colyer 1990). As previously mentioned, the vestigial first premolars are present in nearly 20% of adult horses and are found more frequently in the maxillary than the mandibular arcade (Colyer 1990). Using the Triadan nomenclature system, the 09s in each arcade are the oldest equine permanent teeth, erupting at approximately 1 year of age, whilst the 08s are the youngest, erupting when the horse is approximately 4 years old. The permanent 06s erupt at approximately 2.5 years and the 07s at approximately 3 years of age and permanent cheek teeth are usually in full wear at about 6 months after eruption. The prolonged eruption of equine cheek teeth is due to the constant proliferation and remodeling of the periodontal ligament which creates traction on the tooth, essentially drawing it into the oral cavity (Ten Cate et al 2003). Reserve crowns can be up to 9 cm long at initial eruption of the tooth, and cheek teeth often reach a state of complete senile wear, or 'smooth mouth', at between 20-30 years of age.

1.4.1 Enamel

Enamel, as the hardest of all dental tissues, is formed mostly of hydroxyapatite crystals and is 96% inorganic. It is essentially a dead tissue and, as previously mentioned, cannot repair itself if damaged. As the ameloblasts die when the tooth erupts, the tooth's enamel content is set firmly at the time of eruption and progressively decreases over time with dental wear (Ten Cate et al 2003). In particular, the enamel content of the cheek teeth is lost as the tooth wears, so enamel may not be present in the crowns of some horses over 20 years of age, with the remaining crown being entirely composed of cementum and dentine. Structurally, the enamel layers are thickest parallel to the long axis of the jaw and thinnest where they invaginate into the tooth (Kilic et al 1997a). The occlusal surface of infundibulae in cheek teeth that are in wear appear as cemental 'lakes' surrounded by enamel with a canal, or vascular channel, often present in the centre. Infundibular length, and therefore the length of the enamel 'cup', as a proportion of total crown length was found to significantly change with age, ranging between 99% in a four-year-old horse to 7% in a 16-year-old, with a median value of 82% (Fitzgibbon et al 2010).

1.4.2 Dentine

Dentine is much less hard and brittle than enamel. It is an elastic, avascular tissue that functions in supporting enamel. It is composed of approximately 70% mineralized tissue with a 30% organic content, mainly made up of collagen and water. Although it is avascular, it contains cytoplasmic extensions of the odontoblasts which form during embryonic development and elongate throughout life as secondary dentine is laid down. These cytoplasmic extensions traverse the predentine from the pulp, creating a single functional unit termed the pulpodentinal complex. The dentine is therefore also a sensitive tissue and is capable of repair. As the equine teeth undergo attrition, the primary dentine overlying the pulp horns wears away and subocclusal secondary dentine is continually produced to protect the underlying pulp during dental eruption and wear. The leading occlusal surface, or the aspect of the tooth subject to the greatest masticatory forces, produces the greatest amount of secondary dentine. This corresponds to the lingual aspect of the mandibular cheek teeth and the buccal aspect of the maxillary cheek teeth in the horse. Consequently, the leading occlusal surface also contains the smallest volume of pulp (Dacre et al 2008b; Windley et al 2009a). Tertiary dentine is known as either reactive or reparative dentine, which is produced from existing odontoblasts and undifferentiated mesenchymal pulpal cells, respectively. Tertiary dentine is produced in direct response to trauma or other damage which leads to unintentional pulpar exposure. It lacks much of the normal cellular structure of primary or regular secondary dentine, and essentially acts as a physical barrier to seal the pulp off from noxious stimuli and prevents further damage (Ten Cate et al 2003; Dacre et al 2008b).

1.4.3 Cementum

Cementum is the softest of all dental tissues, containing about 50% mineralized and 50% organic material, mainly collagen and water. It interlocks with enamel at the amelocemental junction at the tooth periphery and also, as noted, should fill the enamel-lined infundibulae in the incisors and maxillary cheek teeth. Infundibular cementogenesis by cementoblasts begins at the periphery of the infundibulae and proceeds circumferentially inwards toward the central vascular channel along the length of the infundibulum. This process is confined to the period prior to and soon after eruption of the tooth (Suske et al 2016a). The cementum also forms a thin protective layer over the peripheral enamel of the entire tooth. Peripheral cementum is much thicker in the gingival portion of the tooth as compared to the alveolar region (Mitchell 2003). Living cementoblasts are found in high concentration within the more vascular gingival region and may even extend several millimeters occlusally onto the clinical crown (Mitchell 2003). While the cementum on the subgingival crown is an active connective tissue with a vascular supply from the periodontal ligament, the cementum of the clinical crown becomes an inert tissue following loss of its vascular supply from the gingival margin (du Toit et al 2008).

1.4.4 Dental Pulp

Dental pulp is a soft, sensitive, and vascular connective tissue contained within pulp horns that lie several millimeters deep to the occlusal surface beneath dentine. The odontoblasts that line its peripheral aspect are responsible for the production of secondary dentine and if necessary, repair with tertiary dentine. The pulp also contains nerves, lymphatics, and blood vessels that serve as the nutrient source for the dentine (Dixon and du Toit 2010). All pulp horns may communicate via a common pulp chamber until up to 15 years post-eruption in mandibular cheek teeth and up to 9 years post-eruption in maxillary cheek teeth, though the common pulp chamber typically begins to divide by 5 years of age. The number of interpulpar communications decreases as the horse ages, though there is much variability in the confirmation of the segmental pulp chambers (Kopke et al 2012). Mandibular cheek teeth typically contain a rostral and a caudal root, with the rostrally and caudally located pulp horns eventually dividing between these roots with some variation. Maxillary cheek teeth have one palatal (medial) and two buccal (lateral) roots. The interpulpar communications within maxillary cheek teeth are more complex and varied than those in the mandibular cheek teeth, and a common pulp chamber is maintained more frequently (Kopke et al 2012). If the maxillary pulp chamber divides, it also typically forms rostral and caudal segments. Increasing age in the horse is associated with decreased pulp volume and extension of the pulp apically within the tooth, though age is not associated with increased segmentation of the pulp chambers once the common pulp has divided (Kopke et al 2012).

1.4.5 Periodontal Tissues

The gingiva is a mucous membrane which immediately surrounds the erupting teeth and is one of the four periodontal tissues. It is termed as masticatory gingiva if it faces the oral cavity or as periodontal gingiva if it faces the tooth. The gingiva protects the underlying periodontal membrane from the oral environment. Any inflammation of the gingiva, or gingivitis, can lead to deeper infection and periodontitis, or inflammation within the periodontal membrane, (Ten Cate et al 2003).

The primary purpose of the periodontal structures is to attach the tooth to the jaw bones and provide this connection with some amount of flexibility. The alveolar process, or the lamina dura denta on radiographs, is a dense layer of alveolar bone attached to the spongy, basal bone of the jaw that contains the teeth. If a tooth is lost and the alveolus is no longer in attached to the cementum, the alveolar bone will degrade over time and become spongy bone, indistinguishable from the remaining bones of the jaws (Ten Cate et al 2003). The periodontal ligament which lies between the tooth and alveolar bone contains fibroblasts along with collagen fibres to maintain the prolonged eruption of the tooth. The periodontal ligament also provides sensory information regarding movement of the tooth within the alveolus via mechanoreceptors. It also provides physical attachment for the tooth within the alveolus via large bundles of these collagen fibres known as Sharpey's fibres (Butler 1991). Once the tooth erupts, no further cementum can be deposited onto the clinical crown. However, cementum may be laid down on the reserve crown or roots as necessary following any insult or trauma to the tooth (Jones 1981).

1.5 Infundibular cemental defects

In theory, all equine cheek teeth infundibulae should be completely filled with cementum from the apex to the occlusal surface. However, this is rarely the case as developmental defects or acquired changes in infundibular cementum are found in 52-90% of infundibulae (Veraa et al 2009; Kilic et al 1997b; Windley et al 2009b; Fitzgibbon et al 2010). This high prevalence of abnormalities has led to a reconsideration of the clinical significance of some of these infundibular lesions in causing more severe dental disease, such as sagittal fractures or apical infection. Windley et al (2009b) termed a normal infundibulum on computed tomographic imaging (CT) as having complete absence of occlusal surface lesions or subocclusal radiolucent areas. Ninety percent of infundibulae showed CT evidence of an abnormality, although only 65% showed significant visible changes occlusally. The prevalence of subocclusal infundibular lesions may increase up to 100% on CT imaging of teeth 6 years or older in dental age, or time since eruption (Windley et al 2009b). A gross anatomical study by Fitzgibbon et al (2010) found 8% of maxillary cheek teeth have lesions visible on the occlusal surface, but 80% of teeth had subocclusal cemental lesions that were visible on gross sectioning of the tooth. Only 12% of the teeth examined had infundibulae that were completely filled with cementum of normal appearance.

1.5.1 Infundibular Caries

Caries is a disease of the calcified tissues of the teeth, whereby the interaction of bacteria and biological substrates, such as dietary carbohydrates, on dental tissues lead to the demineralization of the calcified dental tissues and destruction of their organic components (Soames and Southam 2005). Caries in brachydont species is caused by acid produced in the metabolism of dietary carbohydrates by organisms in the organic pellicle on the surface of the tooth (Martin 2010). Infundibular caries in the horse is clinically recognized as the progressive demineralization of dental cementum, that in some cases may extend to enamel and dentine, which can be seen on the occlusal surface of the tooth (Baker 1970; Colyer 1990; Lundstrom et al 2007). In peripheral caries, plaque on the tooth surface, along with the presence of dietary carbohydrates, allow bacteria to adhere to the tooth and provide them with the nutrition to proliferate. Bacteria such as *Streptococci*, which produce extracellular polysaccharides, can easily adhere to the tooth surface (Borkent and Dixon 2015). Bacterial metabolism leads to increased lactic acid production and a local decrease in the oral cavity pH that can cause dissolution of dental hydroxyapatite crystals, leading to progressive demineralization of the tooth. It is hypothesized that the current increased availability of prepared commercial feed concentrates increases the oral simple carbohydrate content and therefore can allow a decreased oral pH in the horse (Lundstrom et al 2007), although long-term changes in salivary pH have not been found in horses following consumption of molasses-containing feeds (Housewright et al 2003). Although the carbon and fluoride impurities within the enamel along with the calcium and phosphate concentrations within the saliva contribute to the ability of dental tissues to resist erosion, a pH of less than 6.7 for cementum or less than 5.5 for enamel in human teeth can lead to significant demineralization of the respective tissues, particularly in the presence of pre-existing cemental hypoplasia or microfractures which allow bacteria to become readily attached to the weakened surface of the tooth (Dawes 2003). Specific equine cariogenic bacteria have not been isolated, although there appears to be an association with an increase in infundibular caries in horses in Scandinavia and the presence of *Streptococcus devriesei* bacteria (Lundstrom et al 2007). The association is loose though, as these bacteria are part of the normal oral flora in many unaffected horses and may only invade damaged dental tissue following the formation of caries (Collins et al 2004).

Infundibular caries has been reported in up to 97% of equine teeth of dental age, or age since eruption, of 12 years and over, with a much higher prevalence in cheek teeth as opposed to incisors (Honma et al 1962). This is thought to be due to the depth of the infundibulae along with greater enamel ridge formation of cheek teeth allowing for more entrapment and stagnation of food material. Triadan 09 is most affected by caries along with many other recognized dental disorders, such as sagittal fracture which has been attributed to the presence of severe infundibular caries (Fitzgibbon et al 2010; Dixon et al 2014). Caries is frequently classified using the modified Honma scale, in which Grade 0 is an unaffected tooth, Grade 1 caries affect only cementum, Grade 2 caries affect cementum and enamel, while Grade 3 caries involves the surrounding dentine layer, in addition to cementum and enamel. Infundibular caries can progress and allow coalescence of both infundibulae into one large carious central defect in the tooth (Grade 4), which can in turn degrade further and allow the impaction of food and debris into the tooth (Honma et al 1962; Dacre 2005). Dacre developed a modified version of the Honma scale in order to describe cemental hypoplasia and infundibular caries more completely as visualized on CT scan (2005). The numerical grades (0-3) still describe the tissues affected, with 0 being completely normal and 3 progressing to involve cementum, enamel, and dentine. However, the grades are also divided into 2 sub-classifications. Class A teeth show no occlusal surface evidence of infundibular caries, while class B teeth have occlusal carious lesions. Therefore, no class B teeth can be graded as a numerical 0. Dacre found that only 10% of cheek teeth were truly a grade 0, while 65% fell within the ‘B’ classification (Dacre 2005).

Complete (grade 1 or higher) cemental caries can be recognised on CT scan as destruction of the cementum extending the full length of the infundibulum with or without enamel involvement. Complete infundibular cemental caries occur most frequently in horses between 12 and 20 years old, and is 30 times more likely to be found in cheek teeth 109 or 209 than in 111 or 211 (Fitzgibbon et al 2010). The prevalence of cheek teeth infundibular caries varies significantly between different studies and appears to depend on the definition of caries used in individual studies and on the technique used to detect the caries. In some studies, cemental developmental defects including hypoplasia are classified as cemental caries. The reported prevalence of maxillary cheek tooth infundibular caries ranges between 8.7% detected at oral examination (Peters et al 2008), 24% found microscopically (Kilic et al 1997b), and the aforementioned 65% prevalence noted on CT scans. Caries is also more commonly reported in the rostral than the caudal infundibulum, which is thought to be due to earlier loss of the lateral blood supply to the rostral as compared to the caudal infundibulum (Suske et al 2016a), following the breakdown of the dental sac and loss of the main infundibular blood supply upon eruption of the permanent tooth.

1.5.2 Other Cemental Abnormalities

In addition to caries, other recognized infundibular abnormalities include cemental hypoplasia, and other uncategorized cemental defects, such as the presence of discolored or porous cementum (Fitzgibbon et al 2010). On examination of gross dental sections, infundibular cemental hypoplasia was attributed to a persistent central vascular channel within the infundibulae filled with poorly conformed cementum (Dacre et al 2008a). Cemental hypoplasia is recognized as areas of complete developmental absence of cementum with exposure of the enamel in infundibulae, and was present in 22.6% of teeth in one study on longitudinally sectioned samples (Fitzgibbon et al 2010). Previously, it was thought that premature removal of the deciduous premolars was the cause of cemental hypoplasia (Dixon and Dacre 2005), though this is unlikely as the last molar (Triadan 11) is most commonly affected by cemental hypoplasia at the apex of the infundibulum and this tooth does not have a deciduous precursor. Some authors hypothesised that CH may be attributable to loss of blood supply from the apical aspect of the tooth to the infundibular dental organ during development (Fitzgibbon et al 2010), but it is now known that there is no apical blood supply to the infundibulum (Suske et al 2016a). Central infundibular cemental hypoplasia occurs when the central vascular channel fails to fill completely with cementum (Kilic et al 1997b). Junctional cemental hypoplasia occurs at the junction of cementum and enamel along the periphery of an infundibulum (Kilic et al 1997b). Both are typically found midway between the apex and the occlusal surface in the cheek teeth of a young horse, and the areas of hypoplasia typically narrow as they approach the apex of the tooth (Windley et al 2009b). Cemental hypoplasia was rather confusingly defined in a more recent study as a non-pathological developmental abnormality at both the occlusal surface and subocclusal level, and was found at these sites in 51% and 56% of cases, respectively (Suske et al 2016b). However, by definition any deviation from the normal structure or function of a body system is a disease process and therefore is pathological (Newman Dorland 2007). Some cemental hypoplasia is now attributed to lateral vascularization of the infundibulum at the occlusal level. If these vessels allow cementogenesis to be too efficient at the occlusal surface, the cementum produced will occlude the central vascular channel, preventing further cementogenesis more apically (Suske et al 2016a).

As the teeth wear, areas of infundibular cemental hypoplasia, which are thought to be sterile as they are sealed within the developing tooth, may become exposed on the occlusal surface. These areas of hypoplasia then become filled with food that will facilitate bacterial growth and lead to the development of infundibular caries, however the relationship between cemental hypoplasia and infundibular caries is incompletely understood (Fitzgibbon et al 2010). Veraa et al (2009) found that hypoattenuated infundibulae were present in 52% of cheek teeth studied by CT, with all horses having this abnormality in one or more teeth. In addition to the above reasonably well-categorized infundibular abnormalities, generalized cemental defects were found in a further 57.5% of maxillary cheek teeth on study of anatomical sections (Fitzgibbon et al 2010). These abnormalities included localized occlusal surface caries or central linear defects in the cementum along the central vascular channel of the infundibulae. As 88% of infundibulae are not completely filled with normal cementum, variations within the infundibular cemental confirmation may lie within a range of normal morphology that has yet to be defined (Fitzgibbon et al 2010). Misdiagnosis of infundibular lesions is also possible, particularly in young horses. Half of all cheek teeth that have been in wear for less than 2 years actually contain open wide but shallow infundibulae, which form cup-shaped invaginations from the occlusal surface, as a normal anatomical feature (Dacre 2005; Kilic et al 1997a and 1997b). These occlusal surface openings may be mistaken for infundibular caries, but the cementum at the base of the open infundibulum will usually come into wear after several months' without any evidence of a true cemental lesion. However, the cementum at the base of the open infundibulum is at risk of developing caries, as food material may easily become entrapped at this site (Dacre 2005).

There is also some ambiguity in the distinction between cemental hypoplasia and infundibular caries between authors that may explain the large range (24% - 100%) in the reported prevalence of caries (Honma et al 1962 ; Baker 1970; Kilic et al 1997b) which is too wide a range to be accounted for by natural variation in prevalence (Windley et al 2009b). Additionally, the use of more advanced imaging initially led to an over-diagnosis of infundibular caries as many clinically normal maxillary cheek teeth had gas in their infundibulae on computed tomographic imaging, which is now often attributed to cemental hypoplasia. Many cheek teeth have also been shown to have radiolucent areas at the center of the occlusal surface coursing apically that correlate with the central vascular channel. In one CT study, this radiolucent area typically did not extend below the occlusal surface in most cheek teeth, and no further defect was visualized within the infundibulae of these teeth (Windley et al 2009a).

1.6 Sequelae associated with infundibular caries

1.6.1 Periapical Infection

Infundibular caries with erosion of cementum and with food material present near the apex of the infundibulae, were found in 1% of horses in Germany and Sweden (Lundstrom et al 2007). Higher grades of caries (i.e. involving enamel or dentine) are infrequently associated with the presence of pulpar disease as tertiary dentine tends to limit the spread of caries, athough in a minority of cases, progressive destruction of the enamel and dentin that accompanies grade 3 caries can lead to secondary pulpitis and apical infection (Dacre 2005; Dacre et al 2008a). Histological examination has more recently confirmed pulpar infection caused by infundibular caries in 27% of cheek teeth with periapical infection and infundibular caries (Suske et al 2016b). This may be due to the entrance of bacteria from an infected pulp through the apical aspect of the infundibulae (Windley et al 2009b), or from bacterial spread though infundibular enamel into the adjacent dentinal tubules (Suske et al 2016b). Infundibular caries often increase in severity as a horse ages (Honma 1962) while apical infection occurs most commonly in horses aged 4-8 years old (Dixon et al 2000b), suggesting that the two disorders are not commonly linked.

Prior to the recent study by Suske et al (2016b), it was believed that a smaller proportion of infundibular caries was associated with apical infection, with between 5% (Van Enden et al 2008) and 16% (Dacre et al 2008a) of apical infections in maxillary cheek teeth related to extension of caries from one or both infundibulae laterally through the enamel and dentin into a pulpar structure. Furthermore, a direct relationship between lower grade (1-2) infundibular caries and subsequent apical infection in the same tooth has not been fully elucidated, because caries is so frequently present in clinically normal teeth (Veraa et al 2009). Though most do not progress to this extent, infundibular caries weaken the structural integrity of the tooth, particularly grade 3 caries which affect the cementum, enamel, and dentin or grade 4 caries in which the infundibulae begin to coalesce. Coalescing infundibular caries can lead to midline sagittal fracture and apical infection of the tooth, both of which may lead to further complications such as secondary sinusitis.

1.6.2 Cheek Tooth Fracture

In a practice survey (Taylor and Dixon 2007), cheek teeth fractures were recognized in 0.4% of horses on routine dental examination, with 3.4% of affected horses showing signs of apical infection, 33% of horses quidding, and 21% experiencing behavioural problems. Despite these varying signs, 39% of horses were completely asymptomatic. However, Dixon (2000a) found that all referred cases with cheek teeth fractures showed some clinical signs, including quidding, bitting or behavioral problems, or halitosis. Many of these signs were due to periodontal pain from loose dental fragments or from protruding fragments of the remaining tooth that caused lingual or buccal ulceration. Fractured cheek teeth often require exodontia (extraction of the tooth), either of the complete tooth or of loose fragment(s). This procedure often resolves the immediate clinical signs as the pain subsides, but extraction leads to the need for continued and frequent dental management to prevent overgrowths developing on the opposing tooth that can cause severe masticatory abnormalities if neglected. Extraction of the loose fragments is only indicated if there are no clinical signs of apical infection of the fractured tooth (Dixon et al 2007).

Non-traumatic cheek teeth fractures are most frequently longitudinal (sagittal) in orientation, and as noted, are often predisposed to by caries of the cementum and subsequent structural weakening of the tooth, however they appear to be unrelated to senile excavation of older cheek teeth (Dacre et al 2007). In a study by Dixon et al (2000a), 50/400 horses were affected by cheek tooth fracture. Twenty-four of these horses had experienced cheek teeth fractures that were non-traumatic in nature. Longitudinal fractures through the pulp horns or through the infundibulae had been classified as idiopathic cheek tooth fracture (Dixon et al 2000a; Dacre et al 2007), however those related to infundibular caries are no longer classified in this group as the cause has, in fact, been identified. Consequently, midline sagittal maxillary cheek tooth fractures have now been re-classified as ‘infundibular caries-related cheek teeth fractures’ due to the direct relationship found with carious infundibulae and this fracture conformation (Dixon et al 2014). Midline sagittal fractures comprise 23% of all maxillary cheek teeth fractures found in one practice survey (Taylor and Dixon 2007) and 16% seen in a referral centre study, in which they were found to most likely affect Triadan 08-10 maxillary cheek teeth (Dixon et al 2007). Dentinal thickness is reduced in 25% of maxillary cheek teeth that are affected by midline sagittal fractures indicating evidence of previous endodontic disease (Dacre et al 2007).

The maxillary cheek teeth are three times more affected by fractures of any conformation than mandibular cheek teeth, with the Triadan 09s preferentially affected. The reason for the marked prevalence of maxillary 09s to develop midline sagittal fractures is related to the preferential development of severe infundibular caries in this Triadan position (Dixon et al 2014). However, the reason for the high prevalence of other types of (idiopathic) cheek tooth fracture in the maxillary 09 teeth is incompletely understood, as although the teeth are older in dental age than the other teeth but undergo less masticatory force than the more caudal teeth (Dacre et al 2007). In mandibular 09 cheek teeth though, the pre-eruption period is much shorter, which means that there is less time for cementogenesis within the teeth (Hoppe et al 2004), which theoretically should apply to the maxillary 09 teeth as well. Conversely, the prevalence of caries and fractures does not increase in other cheek teeth with increasing age as would be expected as the proportional difference of dental age within the cheek teeth becomes less. Idiopathic cheek teeth fractures can be categorized as either buccal slab fractures through the buccal (lateral) pulp horns, palatal (maxillary) or lingual (mandibular) slab fractures through the medial pulp horns, or atypical slab fractures through other pulpar sites. Buccal slab fractures occur more frequently than midline sagittal fractures, and involve pulp chambers 1 and 2 using the pulpar nomenclature system developed by du Toit (2009). The cause of these idiopathic fractures is obviously unknown, but as noted, some fractured teeth have thinner secondary dentine indicting prior endodontic disease (Dacre et al 2007). Bacterial infection can affect any exposed pulp horns, but in most cases of maxillary slab fracture tertiary dentin seals the affected pulp and prevents apical infection.

In contrast to midline sagittal fractures, 71% of slab fractures are reported to have no gross clinical evidence of apical infection, though a low grade local pulpar and apical inflammatory response may be present that leads to bony alveolar remodeling or granuloma formation (Dacre et al 2007).

Although infundibular caries can extend through the enamel and primary dentin, the secondary dentin within the pulp chambers and the pulp itself typically remain unaffected unless the tooth fractures. The occurrence of midline sagittal fractures increases in horses up to the age of 10 years and then progressively declines as the relative depth of the infundibulae to crown length decreases in older teeth. Aged teeth form cemental deposits around the apices that structurally strengthen the tooth, making them less likely to fracture. (Dacre et al 2007). Approximately 40% of infundibular caries-related maxillary fractured teeth show evidence of occlusal pulpar exposure indicative of pulpar death (Van Enden et al 2008). Midline sagittal fractures almost always involve the entire length of the tooth (Dixon et al 2007). Apical infection is nearly a certainty as the fragments displace, food becomes impacted deep within the fracture, and deep endodontic structures are exposed (Dacre et al 2008a).

1.6.3 Extension of Periapical Infection

Apical infection of maxillary or mandibular cheek teeth can also occur through various other routes. Infection borne in the blood or lympatic system is the most common cause of equine cheek tooth apical infection (Dacre et al 2008a and 2008b). Other less common routes of infection include via severe periodontal disease, spontaneous fissure fractures, or traumatic fractures of the clinical crown that expose the pulp and may also lead to apical infection (Dacre et al 2008a and 2008b). Direct penetration of infection from an infundibulum to the apex through a patent hypoplastic infundibulum that allows food and bacteria from the oral cavity to directly inoculate the apex of the tooth through a wide, patent vascular channel and defect in the infundibular apex has also been identified, though it is an uncommon occurrence (Dacre et al 2008a; Pearce 2015). Chronic cheek tooth apical infection can lead to necrosis and death of all pulp horns along with necrosis and blunting of the roots. Teeth with long-standing infections also frequently experience proliferation of the periodontium and cementum apically, forming a either a granuloma-like or structure or depositing excessive calcified tissue at the roots. Extension of the infection beyond the tooth can destroy the alveolar bone followed by abscessation or draining tract formation into the supporting bones.

Regardless of the inciting cause, apical infection can lead to more severe sequelae such as secondary sinusitis, bone remodeling and facial swelling or draining tracts. Characteristic clinical signs of tooth infection are typically based on the location of their apices. The apices of CT 106-7 and 206-7 typically lie within the maxillary bones. Extension of infection from their respective apices often leads to facial swelling and, in decreasing frequency, formation of a draining tract either to the external surface of the corresponding swelling or into the nasal cavity. CT 108-9 and 208-9 often lie within the rostral maxillary sinus, while 110-11 and 210-11 lie within the caudal maxillary sinus. Infection of these teeth often extends into the overlying sinus cavity, leading to sinusitis (Dixon et al 2000b). If sinusitis is found to be due to extension of apical infection, extraction of the affected tooth is required. If sinusitis persists, more intensive treatment may be necessary including trephination into the sinus, sinoscopy, lavage of the sinus cavities, and possible creation of a sinus osteotomy flap to completely remove exudate related to infection within the sinus cavities (Dixon and O'Leary 2012).

1.6.4 Cheek Tooth Diagnostic Imaging

As effective treatment for fractured cheek teeth and teeth with apical infection usually involves a rather traumatic extraction procedure followed by lifelong dental management, accurate identification of affected teeth and any existing sequelae is essential. Radiography does not give accurate information on many cheek teeth with fractures or early apical infection and diagnosis of periapical infection on the basis of radiography alone has been found to be definitive in only half of all cases (Dixon et al 2000b). This is partly due to the superimposition of multiple and complex head structures on radiographs that often prevents accurate identification of affected teeth (Weller et al 2001). Blinded radiographic reviews by multiple clinicians often produce different interpretations of fractured cheek teeth images (Dacre et al 2007). Another study performed by Dacre (2008b) found that only 63% of maxillary cheek tooth apical infections could be accurately identified radiographically. Even with the advent of more advanced radiographic techniques, a sensitivity of only 50-76% and specificity of 90-95% could be achieved (Weller et al 2001; Townsend et al 2011). If radiographic findings and clinical signs support each other, the accurate diagnosis of a diseased tooth is improved, and the risk of unnecessary or erroneous extraction is reduced. The most definitive radiographic features of apical infection include the presence of periapical radiolucency, periapical halo formation and sclerosis surrounding the tooth, and clubbing of the roots of the tooth (Townsend et al 2011). However, similar radiographic changes may also be present if there is merely apical inflammation, cyst formation, or subclinical infection, which do not necessarily require exodontia (Dixon et al 2007).

Whereas radiographic images can only present a two-dimensional view of the head, computed tomography (CT) can produce three-dimensional reconstructions. Windley et al (2009a) showed that CT imaging was accurately able to image and quantify the length, circumference, and volume of pulp chambers and infundibulae in equine cheek teeth. In a study performed by Veraa et al (2009), apical infection was identified in 29 of 150 (19.3%) of cheek teeth by CT, with 68% of infected teeth showing evidence of incomplete infundibular filling or carious lesions. Alveolar changes were also readily imaged as a widening of the apical periodontal space and increased soft tissue density of the periodontal ligament indicating possible apical infection, however alveolar changes were found to be unrelated to any infundibular defects. These studies emphasize the usefulness of CT in accurate diagnosis and planning of treatment of equine dental disorders. Some degree of infundibular change may be considered within normal limits, particularly when using advanced imaging techniques such as CT imaging. Further gross, histological, and CT image investigations are warranted as the high prevalence of infundibular lesions is in contrast to the low prevalence of clinical sequelae associated with infundibular lesions.

1.7 Aims of this study

As there are currently limited pathological descriptions of maxillary cheek tooth infundibular caries, the aim of this study is to characterize the gross, histopathological, and CT changes in cheek teeth with infundibular caries. These studies may help to differentiate normal anatomical variation, such as innocuous cemental defects, from pathological changes within the infundibula that are more likely to lead to midline sagittal fracture or apical infection of the tooth. It is anticipated that the use of CT will allow a more accurate and complete investigation into the aetiopathogenesis of infundibular caries and in turn will hopefully allow for more conclusive diagnoses and treatment of infundibular disease in the future.

2. Materials and Methods

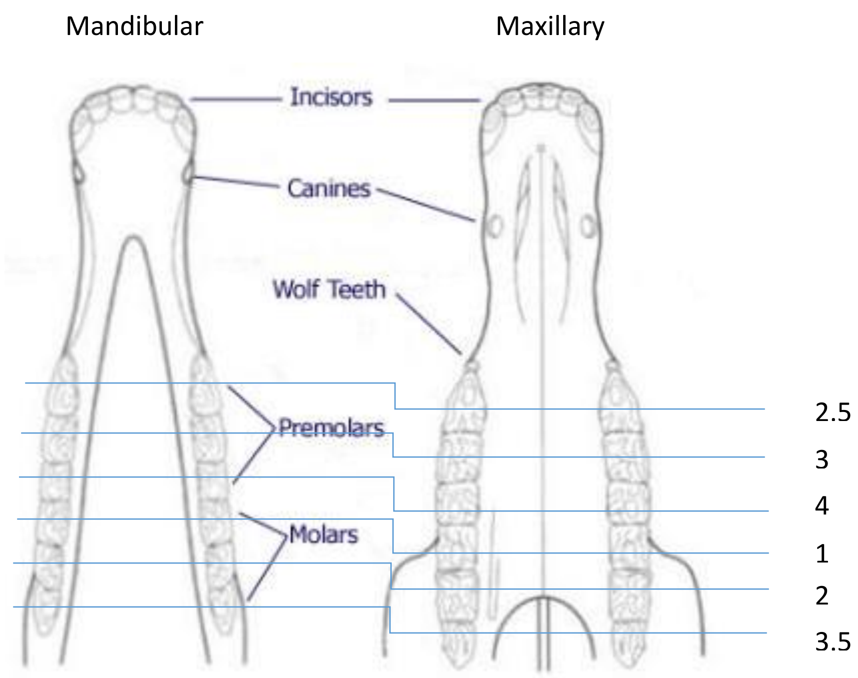
2.1 Sample Collection

All maxillary cheek teeth samples utilised in this study were extracted from cadaver heads following humane euthanasia. The teeth were selected from cases euthanized at the R(D)SVS Equine Hospital for reasons other than dental or sinus disease or from cadaver heads obtained from an abattoir where the reasons for euthanasia were unknown. The horses used for sampling were randomly provided by the abattoir, and those with evidence of secondary complications related to dental disease, such as sinusitis, were excluded. The selected skulls were disarticulated at the temporomandibular joint to allow ease of dental examination and later extraction of selected teeth. Maxillary cheek teeth with other significant dental disorders, such as pulpar exposure indicating apical infection or teeth with fractures were excluded from the study. A total of 100 maxillary cheek teeth and the overlying alveoli were extracted post-mortem from 30 cadaveric heads using a hammer and chisel while attempting to keep the alveoli intact and attached to the intact dental apices (Figure 2.1.1).

Figure 2.1.1: Intact alveolus apical to and surrounding the extracted maxillary cheek tooth

The ages of the euthanized clinical cases were determined by clinical records. The age of the cadavers obtained from the abattoir were estimated by examination of their incisors (Muylle 2010). The age of horses under five years can be relatively accurately determined due to the regular eruption times of the permanent incisor teeth. However, this technique is less accurate as a horse ages. Therefore, to reduce this potential inaccuracy, horse age was recorded in five-year age ranges. In this study, the age of the horse rather than the *dental age* of each tooth was recorded. The permanent teeth erupt sequentially at the following ages (Figure 2.1.2) (Sisson et al 1975):

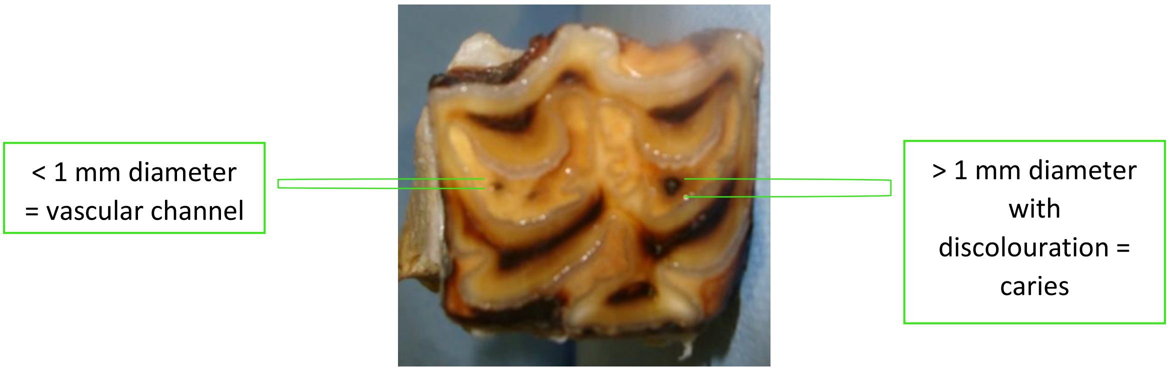
Figure 2.1.2: Eruption age of equine cheek teeth (in years)



2.2 Occlusal Surface Assessment

Infundibulae were assessed as with visible infundibular caries on the occlusal surface or without visible infundibular caries (filled with normal cementum) on occlusal surface examination. Although peripheral caries are another commonly recognised dental disorder in the modern equid, their inclusion is beyond the scope of this study, and therefore further examination or assessment of these was not pursued. In order to differentiate what are termed normal infundibular central vascular channels from localised occlusal infundibular caries, infundibulae were determined to have occlusal surface caries on gross examination if the site of cemental absence surrounding the central vascular channel was greater than 1 mm in diameter and contained discoloured cementum (C Staszyk 2015, personal communications; Suske et al 2016b) (Figure 2.2.1).

Figure 2.2.1: Example of a caudal infundibulum with a prominent central vascular channel (considered normal) on left and with occlusal infundibular caries in the rostral infundibulum as assessed on occlusal surface examination.



After age was determined, we attempted to obtain teeth both with and without visible occlusal lesions from each age group. As a larger number of aged horses tend to be euthanized than younger horses, obtaining an even proportion of samples from each age group was challenging. A larger number of teeth was obtained from a smaller number of young horses due to the difficulty in accessing younger cadavers. Therefore, the effect of these individual horses on the data will be greater than from the older horses from which a small number of teeth was obtained. All cadaver heads were examined by two independent observers prior to sample selection, one by CT scan and one by visual assessment. Teeth with visible occlusal lesions but without any sign of concurrent dental disease (such as pulpar exposure or tooth fracture) were chosen by the independent observers. Most horses examined had at least two teeth with visible infundibular abnormalities on the occlusal surface. Due to this high prevalence of visible infundibular lesions, most (16/18) of the teeth without any visible occlusal surface lesions had to be selected from the same population as those with visible lesions. Eighty-two teeth with infundibular caries were selected along with eighteen teeth with no visible occlusal lesions (100 teeth in total) (Table 2.2.1).

Table 2.2.1: Number of maxillary cheek teeth selected from each skull and whether they had visible occlusal surface lesions or not.

Horse ID	Number of Teeth Selected	Number With Visible Occlusal Lesions	Number Without Visible Occlusal Lesions
102	1	1	0
103	7	5	2
104	5	5	0
105	2	2	0
106	3	3	0
107	2	2	0
108	4	4	0
109	4	3	1
110	4	4	0
111	5	5	0
112	4	3	1
113	4	2	2
114	2	2	0
115	2	2	0
116	2	2	0
117	4	4	0
119	4	4	0
120	4	4	0
121	2	2	0
122	3	3	0
123	2	1	1
124	4	2	2
125	3	3	0
126	2	2	0
127	1	1	0
128	2	1	1
135	2	1	1
136	2	0	2
137	12	7	5
138	2	2	0
Total	100	82	18

Following extraction, the teeth were washed with tap water and the occlusal surface, crown, and apex of each tooth were then photographed. The age of the cadaver and Triadan position of each extracted tooth were recorded. All infundibulae were identified as rostral or caudal and their grade of occlusal caries was recorded. The teeth were then immediately placed in 10% buffered formalin for fixation and storage for the duration of the study.

Occlusal infundibular caries, if present, was graded using the modified Honma grading system (Dacre 2005) on visual examination of the occlusal surface (Figures 2.2.3 and 2.2.4) to describe the dental tissues affected and the severity of the caries:

Modified Honma Equine Caries Grading System:

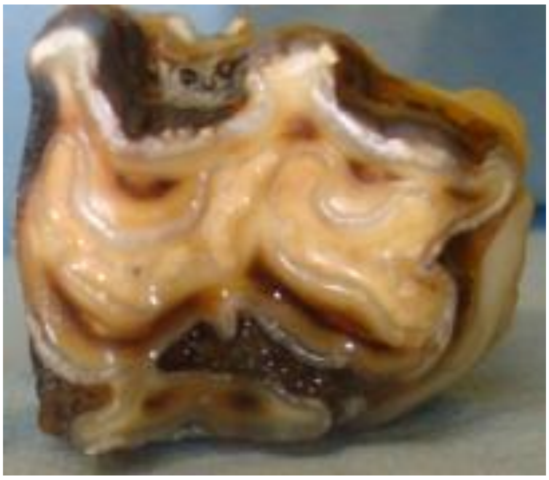
- Grade 1: Confined to cementum
- Grade 2: Extending to adjacent enamel
- Grade 3: Extending to enamel and dentine
- Grade 4: Loss of dental structural integrity

Figure 2.2.3: Occlusal surface appearance of teeth with Grade 1, Grade 2, and Grade 3 infundibular caries in Triadan 09 cheek teeth

Grade 1 | Grade 2 | Grade 3

Figure 2.2.4: Examples of grades of infundibular caries on the occlusal surfaces of selected teeth

Triadan 06 cheek tooth with normal (Grade 0) infundibular cementum
containing no lesion (rostral infundibulum) or a small normal vascular channel (caudal infundibulum)



Triadan 11 cheek tooth with normal (Grade 0) infundibular cementum (note grade 2 peripheral caries – affecting peripheral cementum and enamel)

Triadan 08 cheek tooth with grade 1 caries –only involves cementum (rostral infundibulum - left) and a normal vascular channel (caudal infundibulum - right)

Triadan 10 cheek tooth with grade 1 caries (rostral infundibulum - left) and a normal vascular channel (caudal infundibulum - right)

Triadan 08 cheek tooth with grade 2 caries - involves the cementum and enamel of both Infundibulae

Triadan 09 cheek tooth with grade 1 (left) and Grade 2 (right) infundibular caries

Triadan 09 cheek tooth with grade 3 caries (left) – involves rostral infundibular cementum, enamel and the surrounding (discoloured) dentine and Grade 2 caries (right)

Triadan 09 cheek tooth with grade 2 (left) and Grade 2 (right) occlusal infundibular caries

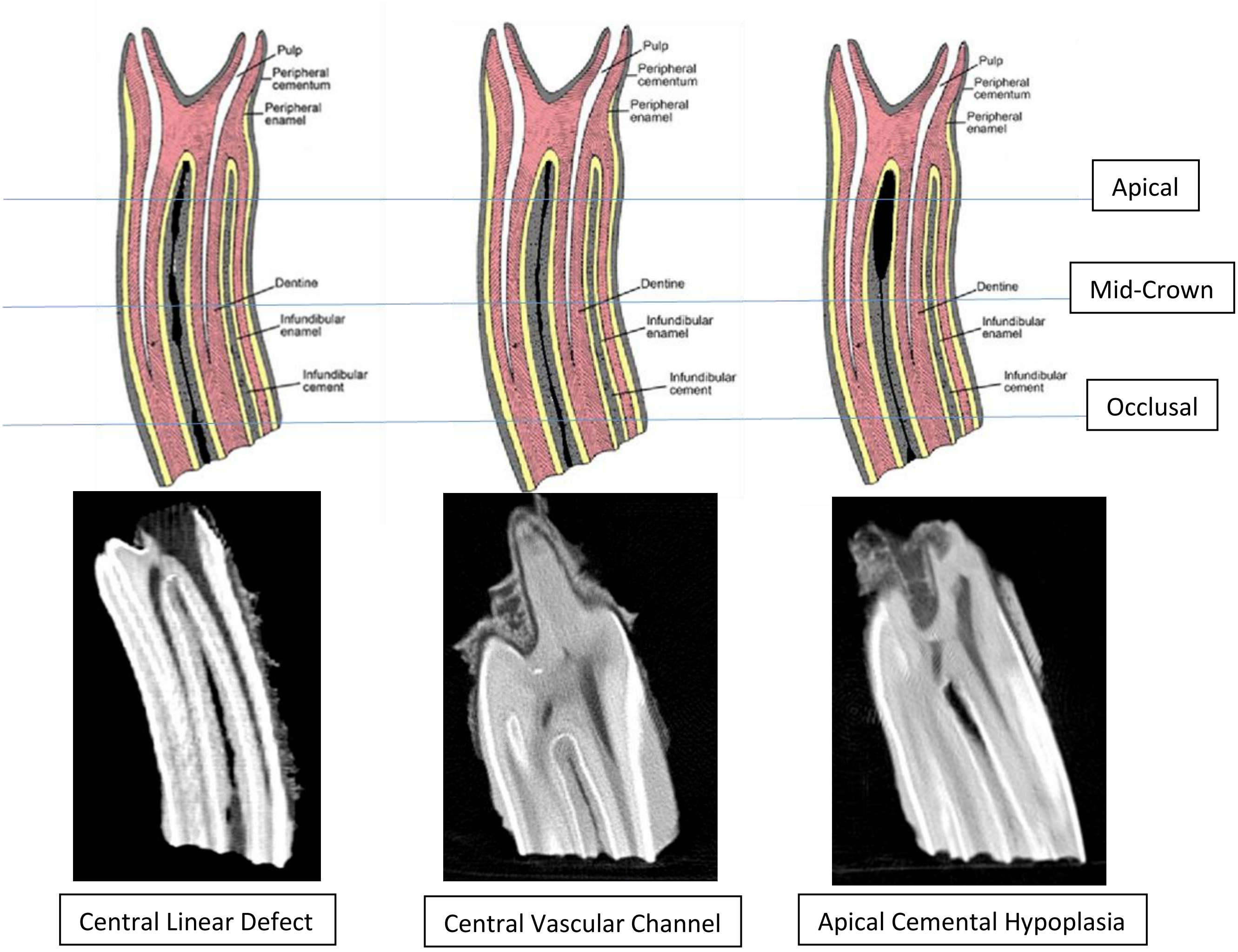
2.3 Computed Tomographic Imaging

Previous gross pathological studies (Fitzgibbon et al 2010) and pilot CT imaging studies (A. Horbal 2014, unpublished observations) had indicated that consistent patterns of lesions occur in cheek teeth infundibulae (Figure 2.3.1) and consequently, for the purpose of this study, these lesions were graded as:

1) Infundibular caries
2) Subocclusal central linear cemental defects
3) Apical cemental hypoplasia

Caries was differentiated on CT scan by the intensity of the material found within the infundibulum, with cemental caries appearing hypointense in comparison to normal cementum but hyperintense in comparison to infundibular areas with defective (partial or total) cemental filling. Central linear cemental defects were recognised as having a linear vertical hypointense area surrounding the central vascular channel that was >1 mm diameter. Cemental hypoplasia was characterised by having a majority of the width of the infundibulum incompletely filled with cementum in a particular section.

Figure 2.3.1: Diagrams of maxillary cheek teeth infundibular cemental patterns along with comparative computed tomographic images. The blue lines in the diagram represent the different locations at which infundibular lesions were classified. The lesions illustrated in the corresponding diagrams and computed tomographic images are listed below the image. A patent central vascular channel is considered a variation in normal infundibular anatomy.



Following initial fixation (1-2 weeks storage in formalin), computed tomographic imaging was performed on all 100 extracted teeth with a multi-slice scanner (Siemens® SOMATOM Volume Zoom, Siemens, Munich, Germany) using a 512 9 512 matrix, 120 kV, 300 mA, at a slice thickness of 0.5 mm with 0.5 mm overlap. The images were later processed with OsiriX© imaging software (Pixmeo SARL, Bernex, Switzerland). Bone window CT data were collected for review and the CT data were transferred as DICOM images to imaging software (OsiriX©). Two- and three- dimensional reconstructions of all teeth were performed using an OsiriX© programme and these reconstructions were used for detailed examinations of each infundibulum and to obtain measurements including:

1) Crown length
2) Depth of rostral and caudal infundibulae
3) Presence of hypointense lesions in infundibulae
4) Depth of lesions in infundibulae classified as:
    a. Occlusal
    b. Mid-crown
    c. Apical
    d. Multiple sites
5) Characteristics of infundibular cemental lesions other than caries
6) Abnormalities of the enamel and dentine surrounding infundibulae with more advanced caries
7) Abnormalities of the apex of the tooth

Any single or combination of lesions at single or multiple locations was recorded.

2.4 Pilot Study of Sectioning and Histology of Cheek Teeth

Four teeth were selected at random from the cadaver samples and sectioned in multiple rostro-caudal planes through both infundibulae using a water-cooled 99-TS230M tile saw with a 20.32 cm diameter 0.081 cm wide diamond-coated lapidary blade (Figure 2.4.1). As the maxillary cheek teeth curve significantly in a palato-buccal direction, and less so in the other two planes, the anatomical features of complete infundibulae could not be followed precisely in the sagittal plane with the rigid saw blade. This necessitated several sections to be taken through each infundibulum in order to assess its most apical aspect. Longitudinal rostro-caudal sections of 3-5 mm width were obtained through the mid-crown portion of several infundibulae also. However, these sections were artefactually damaged by the later described decalcification process, and some anatomical details of these sections were lost. This method was therefore discontinued, with all further sectioning of teeth and any assessment performed in a transverse plane. Transverse, or cross-sections, were obtained at approximately 5 mm intervals along the length of the crown. A visual assessment was made of each section and compared with the corresponding CT scan to compare the appearance of any infundibular lesions found on imaging compared to the appearance of the sectioned tooth on visual examination.

Dental specimens sectioned through the infundibulae were then prepared for light microscopic examination by decalcification using techniques previously described for equine cheek teeth (Kilic 1995). For this process, the sections were removed from the formalin fixative and washed with cold tap water for approximately 20 minutes. They were then placed into Surgipath® Decalcifier II solution (Leica Microsystems Ltd, Milton Keynes, UK) at 20x volume at room temperature. The specimens were frequently checked to establish their degree of calcification and after up to 4 weeks' decalcification, the fully decalcified samples were washed in tap water and then impregnated in Surgipath®Paraplast® Plus tissue embedding medium (Leica Microsystems Ltd, Milton Keynes, UK) and cast into paraffin wax blocks.

Prior to sectioning, the blocks were placed on ice for one hour. Sections of 4-5 micron thickness were then cut on a microtome, floated in a water bath, and then mounted onto coated glass slides. The mounted sections were then dried for 24-48 hours in an incubator at 37°C prior to being stained with haematoxylin and eosin (H&E). All sections were then examined under a light microscope to assess both the quality of the sections and the appearance of the infundibulae, and especially of the infundibular cementum.

Enamel (98% mineral content) was essentially removed entirely via the decalcification process. This process made the teeth less brittle and more amenable to sectioning, but it also made the cementum that should have been held in place by the amelocemental junction more friable and prone to folding, fracturing, or moving on the slide. Several sections had to be recut due to folding or other artefactual damage to infundibular cementum (Figure 2.4.2). Due to this iatrogenic cemental damage caused by the decalcification and sectioning process, especially at the amelocemental junction, quantitative assessment of the infundibular cementum was not considered useful following pilot studies. All histological assessment of infundibular cementum was therefore on a qualitative basis only (under the guidance of Dr S Smith, DACVP, University of Edinburgh, Royal (Dick) School of Veterinary Studies).

Figure 2.4.1: Left: Sectioning of teeth using a lapidary blade and tile saw. Right: 3-5 mm thick longitudinal sections of an older maxillary cheek tooth (with full depth infundibular caries) prior to decalcification.

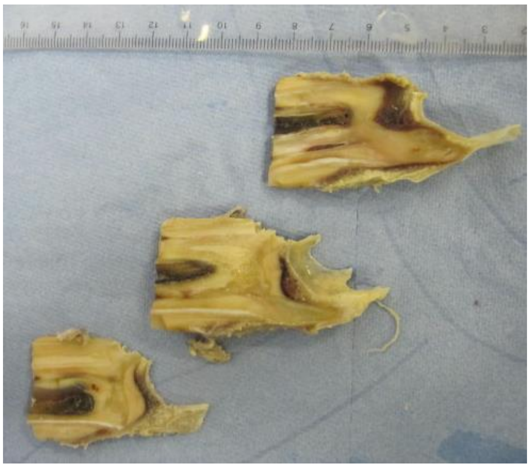
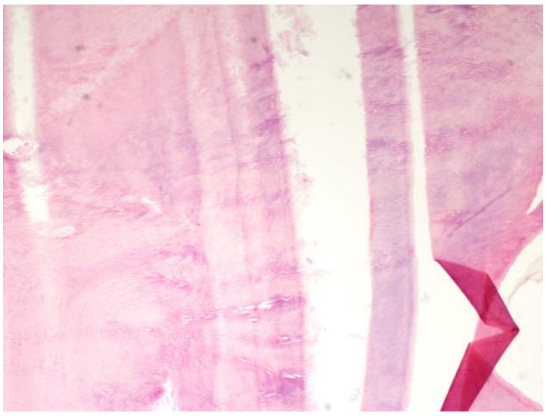


Figure 2.4.2: Left: Thin longitudinal section of a cheek tooth with a central linear cemental defect submitted for histopathology. Right: Folding, fracturing, and processing artefacts on longitudinal sections following decalcification



2.5 MicroCT and Histology

Micro computed tomographic (MicroCT) scanning (XTreme CT, Scanco Medical AG, Bruttisellen, Switzerland; Images courtesy Dr Carsten Staszyk, Justus-Liebig-Universität Gießen, Gießen, Hesse, Germany) with an isotropic spatial resolution of 82 micrometres was performed on 8 cheek teeth that were selected to represent all age ranges (<5, 5-10, 10-15, 15-20, and >20 years) and multiple grades of infundibular caries (Grades 0-3). A rotational x-ray beam with an extremely narrow focus was applied to the tooth from multiple angles and was captured by an x-ray detector. DICOM (Digital Imaging and Communications in Medicine) software then utilized this information to create cross-sectional images of the object in multiple planes, which could be used to reconstruct the tooth in three dimensions. The dental images could then be examined in different planes on a computer using OsiriX© imaging software, and individual sections could be examined in detail.

Qualitative analysis of the microCT images was performed to identify areas of interest in the infundibulae, particularly areas of suspect linear cemental defects or apical cemental hypoplasia. Areas with apparently porous infundibular cementum were also recorded, along with sites of disruptions in the enamel and dentine adjacent to areas of carious infundibular cementum.

Quantitative analysis included measurement of the subocclusal depth of any noted infundibular lesions. Tissue attenuation of the infundibular cementum and all infundibular lesions was measured in Hounsfield Units (HU) utilising an ROI measurement tool on sagittal two-dimensional image sections using OsiriX© imaging software.

The identification of lesions and their sites within infundibulae on microCT images was utilized to select sections for histology. The teeth were sectioned in transverse planes as described previously. Sectioning the maxillary cheek teeth in transverse planes allowed the sections to be unaffected by the curvature of the teeth, enabling the areas of interest identified on microCT to be accurately targeted. Dental slices of 3 mm or greater thickness were obtained from selected sites and these transverse tooth sections were examined visually and photographed. Selected sections were then decalcified and prepared for histology as described above.

All histological sections were then examined by light microscopy (Olympus BX41 Microscope, Olympus KeyMed Ltd, Essex, UK), with areas of interest being imaged with attached camera software (Cell^D©, Olympus Soft Imaging Solutions GmbH, Olympus Europe). As noted previously, infundibular cementum was examined on a qualitative basis only, documenting the intra-infundibular sites and the nature of its lesions. The characteristics of any material contained within these defects, including plant material or damaged and fragmented cementum was noted. The number, sites, sizes and orientation of vascular channels within infundibular cementum were characterised, and a higher power microscopic examination of these sites was made to detect possible endothelial lining cells to confirm the possible presence of previous vasculature.

Any sections found to contain red or brown stained infundibular cementum were of particular interest as we hypothesised that they may contain hemosiderin. With any such finding, further sections were cut from the previously prepared paraffin wax blocks and stained with Prussian blue stain and re-examined using light microscopy. This stain highlights any iron in tissues and would identify red blood cells or their breakdown products, such as haemosiderin, within the infundibulum. In addition to examination of cementum, the space around the infundibular cementum where the enamel was decalcified and lost was examined for any enamel remnants or changes to the periphery of the infundibular cementum and adjacent (more peripheral) dentine. Any abnormalities visualized on histological examination of the remainder of the tooth, including the pulp chambers and the apex, were also recorded.

2.6 Statistical Techniques

This study was designed as a cross-sectional survey. By nature of this observational study, many factors were outside the knowledge or control of the observer, and any conclusions regarding prevalence in the equine population as a whole must be interpreted accordingly. Additionally, bias in the sample selection techniques also limits the application of this survey data to the general population. Most continuous data obtained were non-normal in distribution, determined by use of probability plot analysis (Minitab 17.1.0®, Minitab Inc, State College, Pennsylvania, USA) and consequently were analysed using non-parametric analysis, typically using Mann-Whitney and Wilcoxon Signed Rank tests. For the majority of qualitative data analysis, McNemar's and Chi-square test were used to analyse nominal data while the Kruskal-Wallis and Mann-Whitney tests were used to analyse ordinal data. Most statistical calculations were made using Minitab 17.1.0® software and Stata 14© (StataCorp LP, College Station, Texas, USA), with McNemar's tests performed using GraphPad® (GraphPad Software Inc, La Jolla, California, USA).

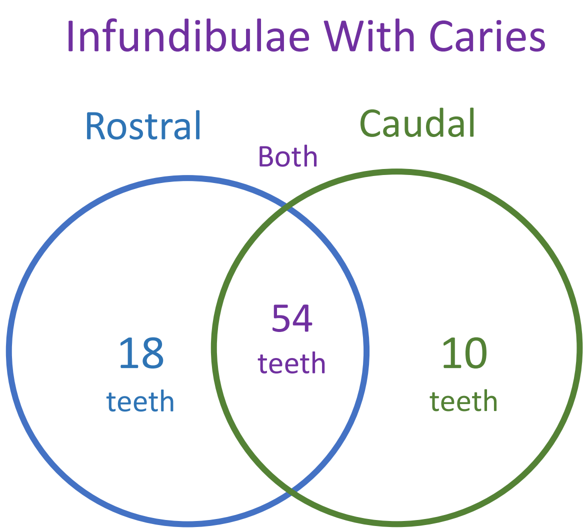
3. Results

3.1 Occlusal Surface Assessment

3.1.1 Caries Prevalence

One hundred maxillary cheek teeth, each continuing 2 infundibulae, were grossly examined on their occlusal aspect and then extracted from 30 cadaver skulls. Occlusal infundibular caries was present in at least one infundibulum in 82 teeth while 18 teeth were not affected by occlusal infundibular caries. In the 82 teeth with occlusal caries, both infundibulae had visible occlusal caries in 54 teeth. In the remaining 28 teeth, the rostral infundibulum alone had visible caries in 18 teeth and the caudal infundibulum alone in 10 teeth (Figure 3.1.1).

Figure 3.1.1: Distribution of occlusal infundibular caries between the rostral, caudal, or both infundibulae of teeth presenting with occlusal infundibular caries



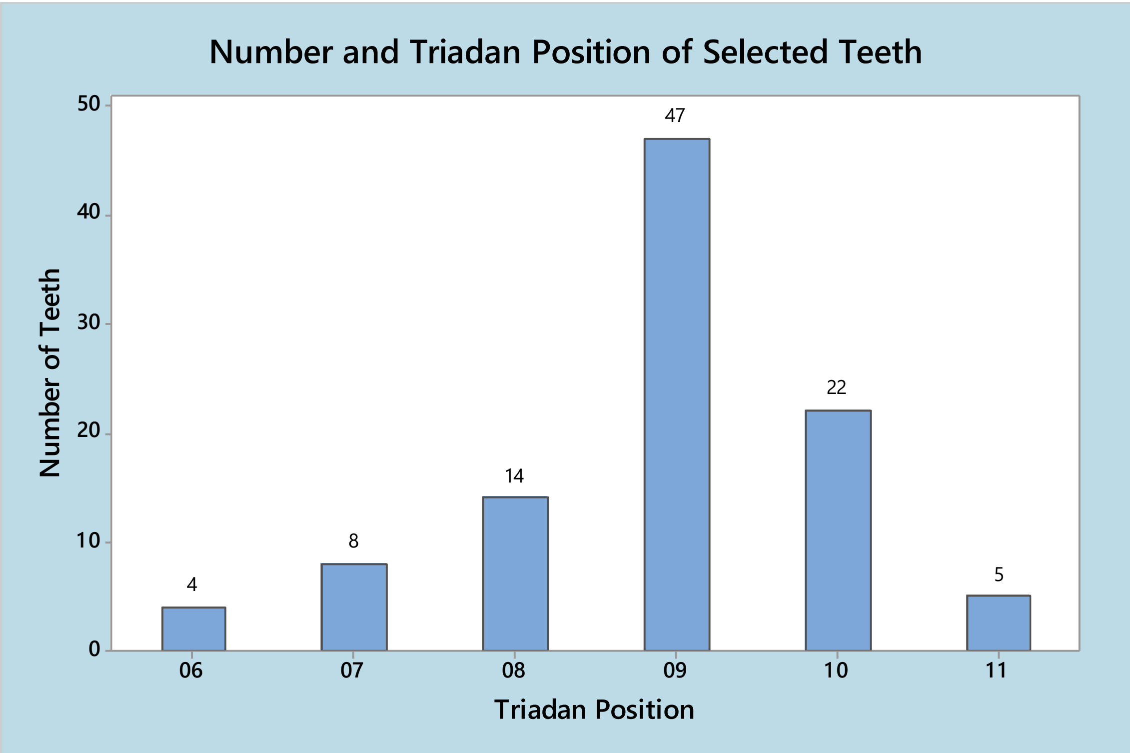
Although, the rostral infundibulae were more commonly (72%) affected than caudal infundibulae (64% affected), this difference was not significant (McNemar's test; $p = 0.1859$) (Table 3.1.1).

Table 3.1.1: Prevalence of rostral and caudal infundibular occlusal infundibular caries lesions in 100 CT.

Infundibulum	Rostral With Caries	Rostral Without Caries	Total Teeth
Caudal With Caries	54	10	64
Caudal Without Caries	18	18	36
Total	72	28	100

The 100 teeth selected included maxillary CT from every Triadan cheek tooth position (06-11) (Figure 3.1.2). The 09 cheek teeth were greatly over-represented (n=47), while the 06 and the 11 positions (n=4 and n=5 respectively) were under-represented.

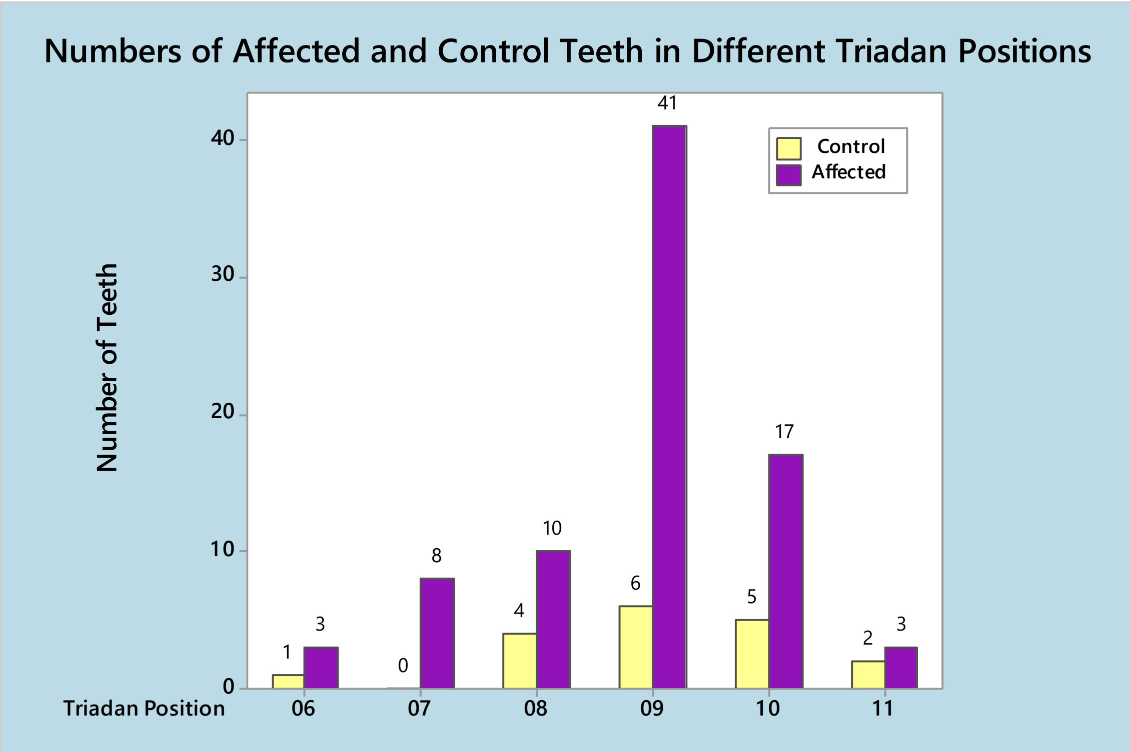
Figure 3.1.2: Number and Triadan position of 100 selected maxillary cheek teeth.



Relationship between occlusal infundibular caries and Triadan position

Of the 82 teeth with visible caries, 41 (50%) were in the Triadan 09 position while only 3 (3.5%) were obtained from both the 06 and 11 Triadan positions. The highest number of teeth without visible occlusal lesions (n=6; 33%) were also obtained from the 09 position, while none were obtained from the 07 position (Figure 3.1.3).

Figure 3.1.3: Number and Triadan position of 82 teeth with occlusal surface infundibular caries and 18 teeth without visible occlusal lesions.



While 40% (n=2) of the 5 Triadan 11 teeth were without visible occlusal surface lesions, 13% (n=6) of the Triadan 09 teeth were without visible occlusal surface lesions and all of the 07 teeth had visible occlusal lesions. Details of the teeth with and without occlusal surface lesions in each Triadan position are given in Table 3.1.2.

Table 3.1.2: Distribution of teeth with and without occlusal infundibular caries by Triadan position in 100 maxillary cheek teeth.

TRIADAN POSITION	06	07	08	09	10	11	TOTAL TEETH
WITH CARIES	3	8	10	41	17	3	82
WITHOUT CARIES	1	0	4	6	5	2	18
TOTAL	4	8	14	47	22	5	100
% UNAFFECTED	25	0	29	13	23	40	18

The teeth were selected to include samples from all age groups (Figure 3.1.3) without any selection for Triadan position. The least number of samples (n=14), were obtained from horses <5 years in age while the highest numbers (n=27) were obtained from horses in the 5-10 years old age group.

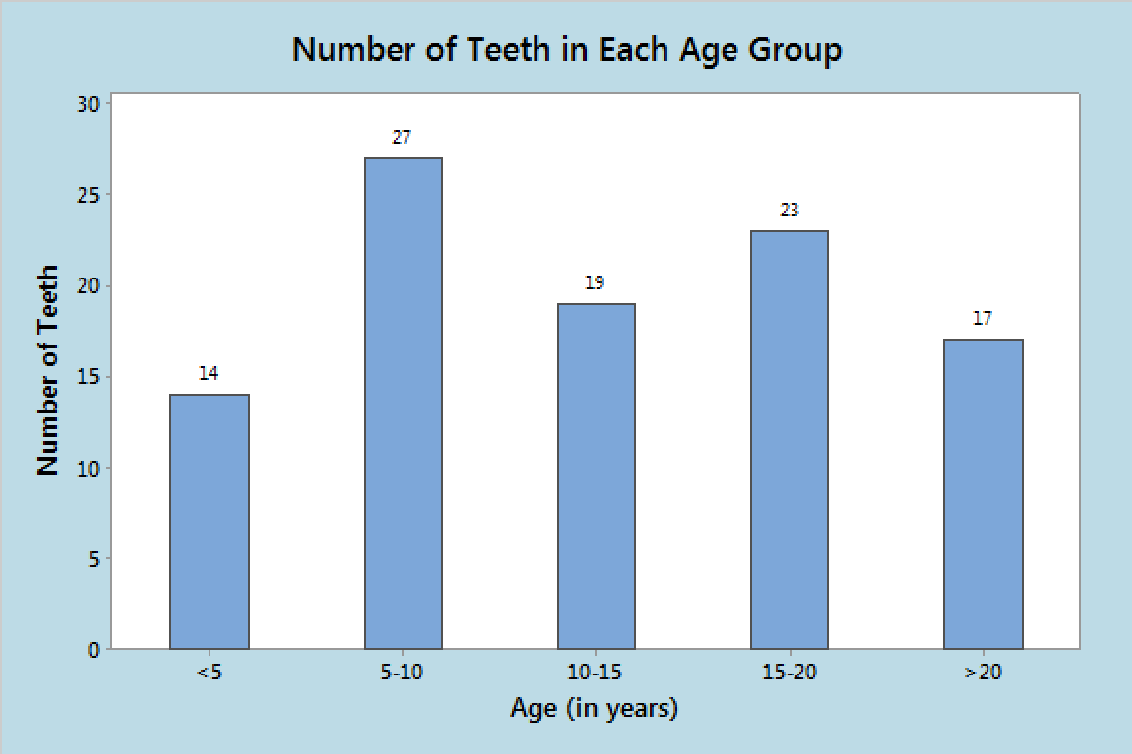
There was a significant relationship between the presence of occlusal infundibular caries and Triadan position (p = 0.007; Chi-Squared Test), ranging from 88% prevalence in the 08 position to 30% in the Triadan 11 position. (Table 3.1.4). When the Triadan positions with the least number of samples (06 and 11) were removed from the analysis, the relationship between prevalence of occlusal infundibular caries and the remaining Triadan positions remained significant (p = 0.027; Chi-Squared Test).

Table 3.1.4: Distribution of infundibulae with visible occlusal caries and without caries by Triadan position in 100 maxillary cheek teeth.

TRIADAN POSITION	06	07	08	09	10	11	TOTAL
CARIES	5	14	16	72	26	3	136
NO CARIES	3	2	12	22	18	7	64
TOTAL	8	16	28	94	44	10	200
% AFFECTED	63	88	57	77	59	30	68

Relationship between occlusal infundibular caries and age group

Figure 3.1.3: Number of selected maxillary cheek teeth in each age group



Of the 200 infundibulae examined in the 100 selected cheek teeth, a total of 136 were affected by occlusal surface infundibular caries (68% of infundibulae) with 32% (64/200) unaffected on the occlusal surface.

Due to age-related bias in tooth selection, statistical analysis of age-related prevalence was not possible. The distribution of infundibulae with and without occlusal surface caries in the different age groups is presented in Table 3.1.3.

Table 3.1.3: Distribution of infundibulae with and without visible occlusal caries in the different age groups in 100 maxillary cheek teeth.

AGE (YEARS)	< 5	5-10	10-15	15-20	> 20	TOTAL
CARIES	17	32	32	32	23	136
NO CARIES	11	22	6	14	11	64
TOTAL	28	54	38	46	34	200
% AFFECTED	61	59	84	70	68	68

3.2 Occlusal Infundibular Caries Grade

3.2.1 Prevalence of occlusal surface caries grade

Of the 136 infundibulae with occlusal caries, most (n = 84; 61.8%) had Grade 1 caries, with Grade 2 (n = 38; 27.9%) and Grade 3 infundibular caries (n = 14; 10.3%) less commonly recorded (Figure 3.2.1 and 3.2.2).

When the rostral and caudal infundibulae were paired by tooth and the differences in their caries grade tested, the rostral infundibulae were significantly more frequently affected by a higher grade of caries than the caudal infundibulae, ($p = 0.02$; Wilcoxon Signed Rank Test, Wilcoxon Statistic = 816.0). A summary of the difference in grade of infundibular caries between rostral and caudal infundibulae is presented in Figure 3.2.3. Most teeth (n=52; 52%), were affected by caries of the same grade in their two infundibulae, including two Grade 0 (without visible occlusal caries) infundibulae. However, in the 48 teeth with a difference in caries grade between their two infundibulae, the rostral infundibulum was affected by caries one grade more severe than of the caudal infundibulum in 29 teeth (41.7%).

Figure 3.2.1: Grade of occlusal infundibular caries present in 200 maxillary cheek teeth infundibulae (Grade 0 = without visible occlusal caries)

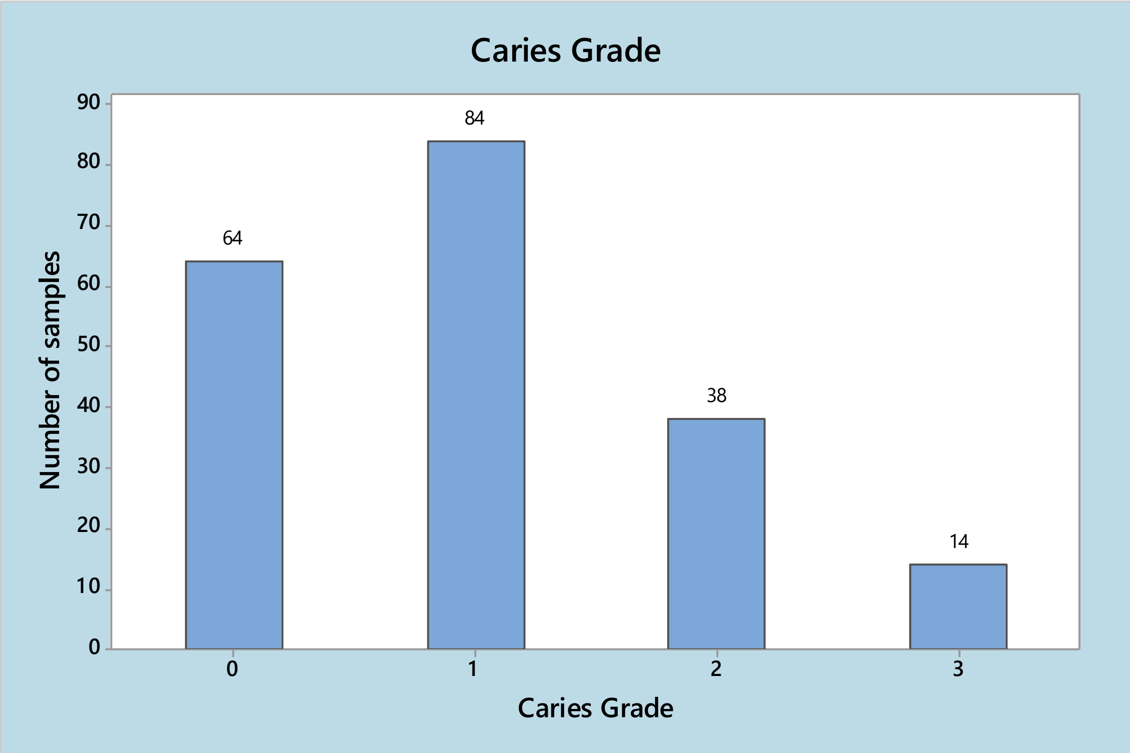


Figure 3.2.2: Distribution of grade of occlusal infundibular caries in 100 rostral (left) and 100 caudal (right) maxillary cheek teeth infundibulae (Grade 0 = without visible occlusal caries).

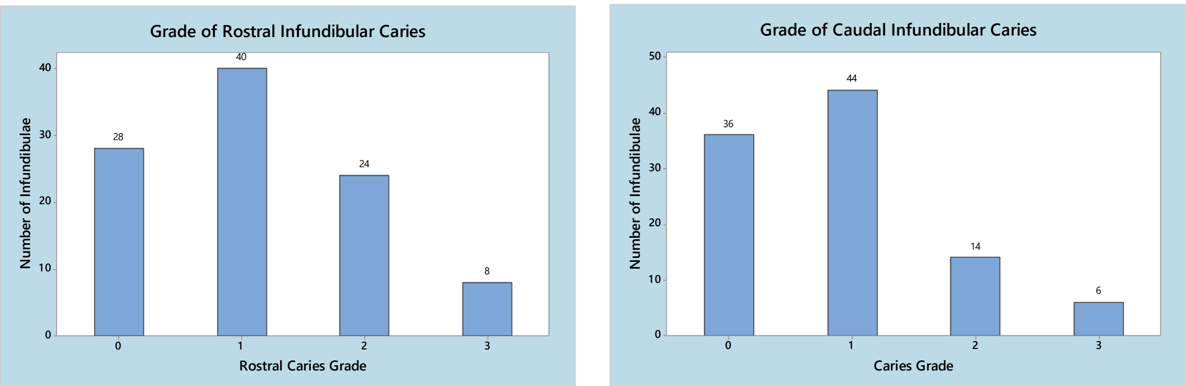
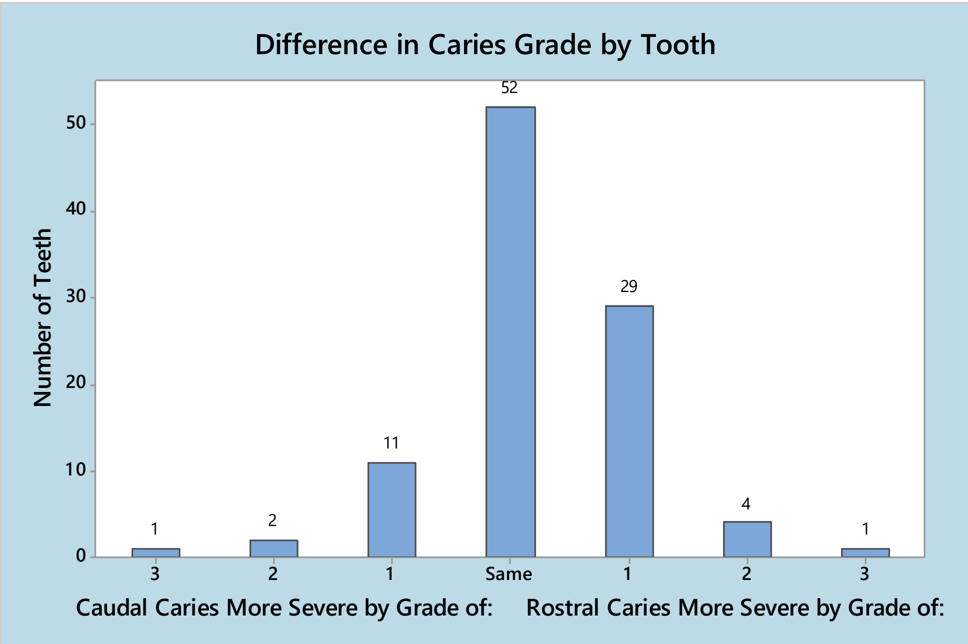


Figure 3.2.3: Difference in occlusal caries grade between the rostral and caudal infundibulae in 100 teeth. The 'Same' bar in the centre of the graph indicates that the rostral and caudal infundibulae of each tooth were affected by the same grade of caries, including Grade 0 (without visible occlusal caries). To the left of the 'Same' bar, the caudal infundibulum was more severely affected than the rostral infundibulum by either 1, 2, or 3 caries grades. To the right of the 'Same' bar, the rostral infundibulum was more severely affected than the caudal infundibulum by either 1, 2, or 3 caries grades. As expected from the above findings, the rostral infundibulae are more commonly affected by caries than the caudal infundibulae, and when present, rostral infundibular caries is usally of a more severe grade than the caudal infundibular caries.



Tables 3.2.1 shows the number of infundibulae affected by each grade (0-3) of occlusal caries in each age group. The proportion of infundibulae affected by caries grade in each age group is presented numerically in Table 3.2.2 and also as a histogram in Figure 3.2.4.

Relationship between grade of occlusal infundibular caries and age group

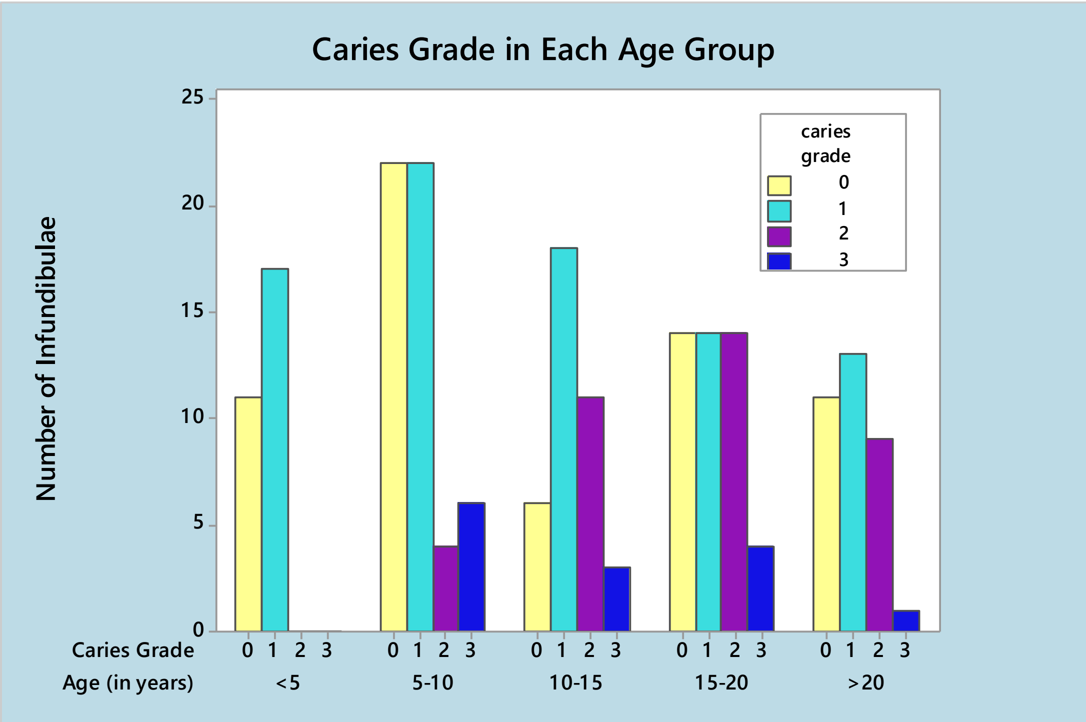
Table 3.2.1: Distribution of occlusal infundibular caries grade in 200 maxillary cheek teeth infundibulae in each age group (Grade 0 = without visible occlusal caries).

AGE (YEARS)	< 5	5-10	10-15	15-20	> 20	TOTAL
GRADE 0	11	22	6	14	11	64
GRADE 1	17	22	18	14	13	84
GRADE 2	0	4	11	14	9	38
GRADE 3	0	6	3	4	1	14
TOTAL	28	54	38	46	34	200

Table 3.2.2: Proportion of infundibulae affected by different grades of occlusal infundibular caries grade in each age group (Grade 0 = without visible occlusal caries).

AGE (YEARS)	< 5	5-10	10-15	15-20	> 20	TOTAL
GRADE 0	40%	41%	16%	30%	32%	32%
GRADE 1	60%	41%	47%	30%	38%	42%
GRADE 2	0%	7%	29%	30%	26%	19%
GRADE 3	0%	11%	8%	9%	3%	7%

Figure 3.2.4: Proportion of infundibulae affected by different grades of occlusal infundibular caries grade in each age group (Grade 0 = without visible occlusal caries).



There was a statistically significant association between caries grade and age group (p = 0.012; Kruskal-Wallis Test) with more grade 2 infundibular caries recorded in the three older as compared to the two younger age groups.

Relationship between grade of occlusal infundibular caries and Triadan position

Tables 3.2.3 and 3.2.4 show the numbers and proportions, respectively, of infundibulae affected by each caries grade (0-3) in each Triadan position; the former data are also presented as a histogram in Figure 3.2.5.

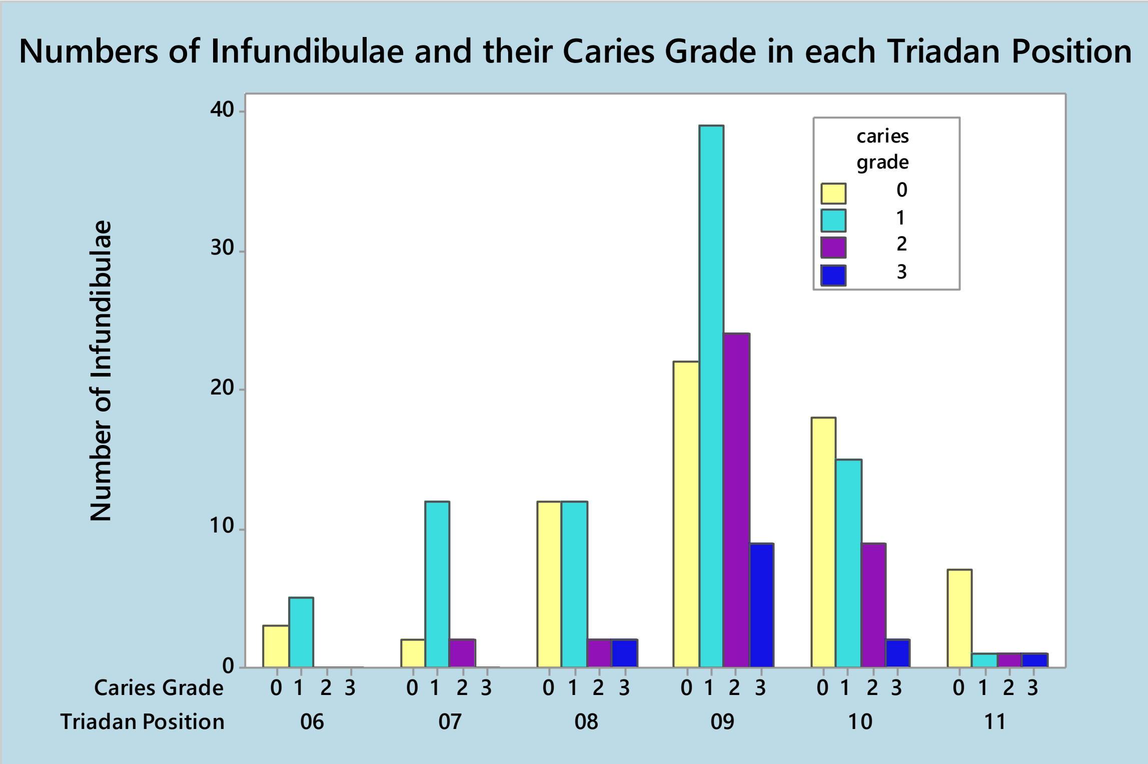
Table 3.2.3: Distribution of occlusal infundibular caries grade (Grade 0-3) present in 200 infundibulae classified by Triadan position (Grade 0 = without visible occlusal caries).

TRIADAN POSITION	06	07	08	09	10	11	TOTAL
GRADE 0	3	2	12	22	18	7	64
GRADE 1	5	12	12	39	15	1	84
GRADE 2	0	2	2	24	9	1	38
GRADE 3	0	0	2	9	2	1	14
TOTAL	8	16	28	94	44	10	200

Table 3.2.4: Proportion of teeth affected by different grades of occlusal infundibular caries in each Triadan position (Grade 0 = without visible occlusal caries).

TRIADAN POSITION	06	07	08	09	10	11	TOTAL
GRADE 0	38%	13%	43%	23%	41%	70%	32%
GRADE 1	62%	75%	43%	41%	34%	10%	42%
GRADE 2	0	13%	7%	25%	20%	10%	19%
GRADE 3	0	0	7%	10%	5%	10%	7%

Figure 3.2.5: Distribution of grades of occlusal infundibular caries between each Triadan position (Grade 0 = without visible occlusal caries).



There was a statistically significant correlation between the grade of occlusal infundibular caries present and the Triadan positions of the 100 examined teeth (p = 0.012; Chi-Squared Test).

3.3 Computed Tomography Findings

3.3.1 Infundibular Depth and Crown Length Measurements

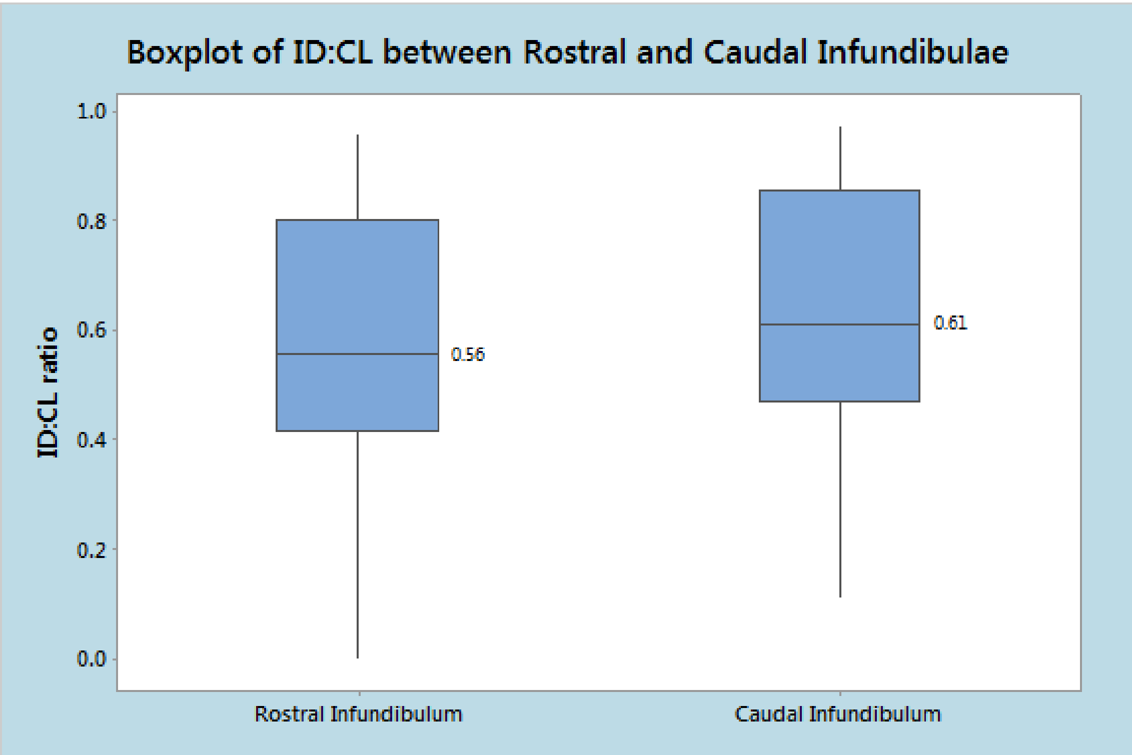
Measurements of total crown length and each infundibulum's depth were compiled from standard (helical) CT scans. The mean and median crown length of all teeth, depth of all infundibulae, and ratio of infundibular depth:crown length are reported in Table 3.3.1. Both of these measurements along with the infundibular depth:crown length ratio are distributed non-normally, indicating non-parametric analysis was most appropriate to assess their association with other variables.

Table 3.3.1: Mean and median crown length, infundibular depth, and infundibular depth:crown length (ID:CL) ratio in 100 maxillary cheek teeth

	MEAN	MEDIAN	MINIMUM	MAXIMUM	STANDARD DEVIATION
CROWN LENGTH (CM)	5.04	4.64	2.14	8.21	1.72
INFUNDIBULAR DEPTH (CM)	3.30	2.56	0	7.44	2.06
ID:CL RATIO	0.60	0.59	0	0.97	0.23

The median ID:CL ratio of the rostral infundibulum was 0.56, while the median ID:CL ratio of the caudal infundibulum was 0.61, indicating that the caudal infundibulum comprised a slightly higher proportion of dental crown length than the rostral infundibulum, and this difference was statistically significant ($p = 0$; Wilcoxon Signed Rank Test, Wilcoxon Statistic 934.0). There was a slightly larger spread of the rostral infundibular ID:CL ratio as opposed to the caudal infundibular ID:CL ratio (Fig 3.3.1).

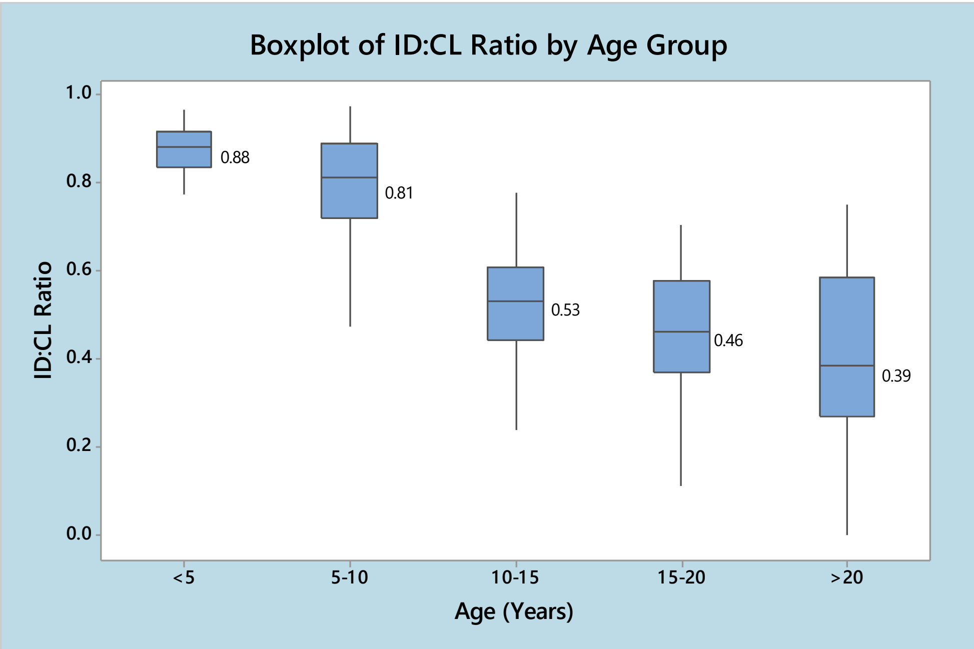
Figure 3.3.1: Boxplot showing median value and interquartile range of the ID:CL ratio in the rostral and caudal infundibulae of 100 maxillary cheek teeth. The median ID:CL ratio is displayed to the right of the respective interquartile ranges.



Relationship between ID:CL ratio and age group

The infundibulum occupied a maximal (median 88%) proportion of crown length in horses <5 years in age and gradually reduced to a median of 41% of crown length in horses >20 years in age (Figure 3.3.2). This age-related reduction in ID:CL ratio was significant (p = 0; Kruskal-Wallis Test).

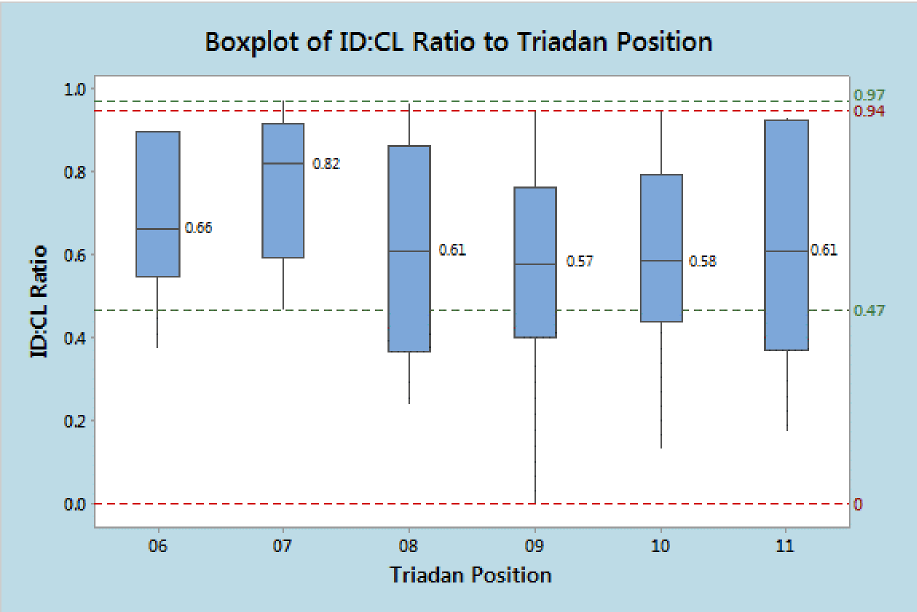
Figure 3.3.2: Boxplot showing median value and interquartile range of the ID:CL ratio in different age groups



Relationship between ID:CL ratio and Triadan position

The ID:CL ratio also was significantly different between the Triadan positions of the cheek teeth (p = 0.033; Kruskal-Wallis Test), with the median Triadan 09 ID:CL ratio being the lowest at 0.57 and the median Triadan 07 proportion being the greatest at 0.82 (Figure 3.3.3). The interquartile range of the Triadan 09 ID:CL ratio was also the largest (0.94), while the interquartile range of the Triadan 07 ID:CL ratio was the smallest (0.50). This difference in data spread may account for some of the difference in the ID:CL ratio of these Triadan positions.

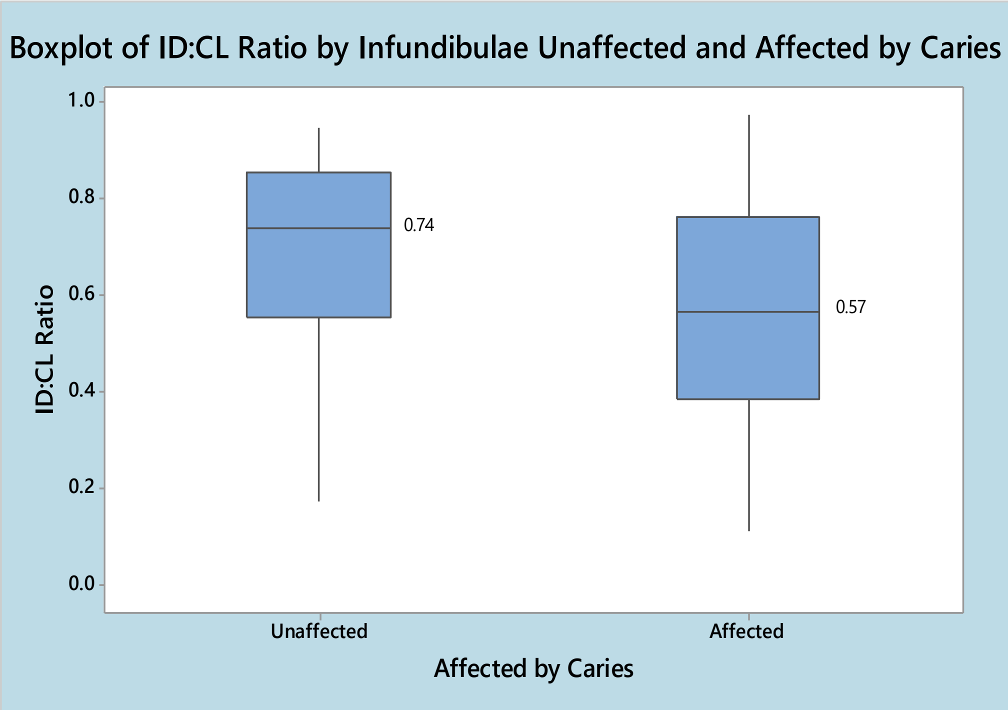
Figure 3.3.3: Boxplot showing median value and interquartile range of differences in ID:CL ratio between Triadan positions. The median ID:CL proportion of each Triadan position is depicted beside the interquartile range. The red dashed lines indicate the range of the largest ID:CL ratio (Triadan 09) while the green dashed lines indicate the range of the smallest ID:CL ratio (Triadan 07).



Relationship between ID:CL ratio in infundibulae with and without visible occlusal caries

The difference between the median ID:CL ratio of infundibulae affected by occlusal caries (0.57) and the median ratio of infundibulae without visible occlusal caries (0.74) was statistically significant (p = 0; Mann-Whitney Test) (Figure 3.3.4). The overall prevalence of occlusal infundibular caries increased significantly as the ID:CL ratio becomes smaller. The high number of 09 teeth included in this study, with their predisposition for infundibular caries along with their significantly lower ID:CL ratio (as they erupt first) likely contribute to this finding.

Figure 3.3.4: Boxplot showing median value and interquartile range of ID:CL ratios in infundibulae with and without visible occlusal caries.



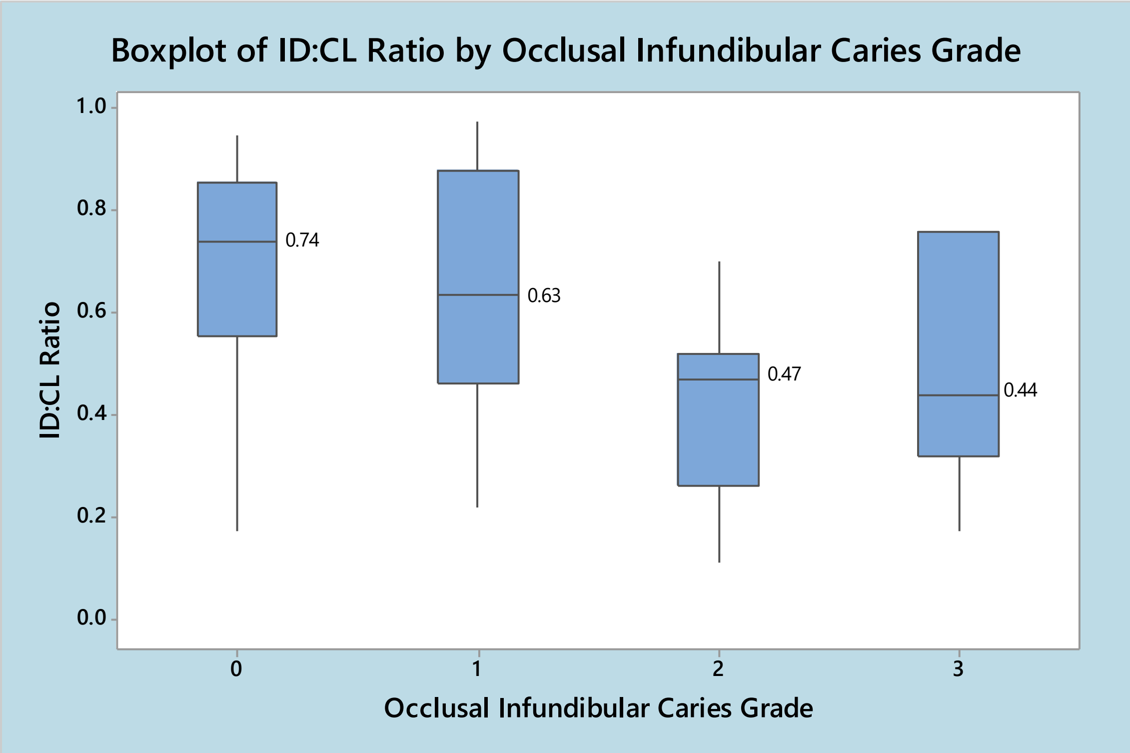
Relationship between ID:CL ratio and grade of occlusal infundibular caries

There was a statistically significant difference between the medians of the ID:CL ratio of cheek teeth with different grades of occlusal infundibular caries ($p = 0$; Kruskal-Wallis Test) (Table 3.3.2). The ID:CL ratio decreased as caries grade increased, from a median of 0.74 in infundibulae without visible occlusal caries, to a median of 0.44 in infundibulae with visible Grade 3 occlusal caries (Figure 3.3.5), likely reflecting age-related increased prevalence in severe caries and the predisposition of Triadan 09 teeth to have caries.

Table 3.3.2: Numbers and median ID:CL ratios of infundibulae with different grades of occlusal infundibular caries (Grade 0 = unaffected).

CARIES GRADE	N	MEDIAN ID:CL
GRADE 0	64	0.74
GRADE 1	84	0.63
GRADE 2	38	0.47
GRADE 3	14	0.44
TOTAL	200	

Figure 3.3.5: Boxplot showing median value and interquartile range of ID:CL ratios of 200 infundibulae with different grades of occlusal caries (Grade 0 = unaffected).



3.3.2 Computed tomographic examination of cheek teeth

Both infundibulae of each tooth were examined for lesions using longitudinal and transverse CT scans. Infundibular lesions noted included more occlusally located lesions, which would also be visible on gross visual examination and deeper infundibular lesions only visible on CT scans, hereafter termed subocclusal lesions. These examinations showed that 182/200 (91%) of infundibulae and 96/100 (96%) of teeth to be affected by at least one infundibular lesion. Both infundibulae had at least one lesion in 86% (86/100) of teeth, one infundibulum had at least one lesion in 10% of teeth and only 4% of teeth had no infundibular lesions.

On CT examination, the rostral infundibulae had lesions in 94% of teeth, while the caudal infundibulae had lesions in 88% of teeth. There was no significant difference between the prevalence of these rostral and caudal infundibular lesions (McNemar's test; $p = 0.114$) (Table 3.3.3).

Table 3.3.3: Numbers of rostral and caudal infundibulae with (affected) and without (unaffected) computed tomographic-detected lesions in 100 maxillary cheek teeth.

Infundibulum	Rostral Affected	Rostral Unaffected	Total Number of Teeth
Caudal Affected	86	2	88
Caudal Unaffected	8	4	12
Total	94	6	100

Relationship between subocclusal infundibular lesions and Triadan position

Due to the high prevalence of infundibular lesions (86%-100% prevalence in 5 Triadan positions) (Table 3.2.2), statistical analysis to examine for differences in the prevalence of lesions between Triadan positions was not possible. Only 5 CT from the Triadan 11 position were examined, with only 2 (40%) having a subocclusal infundibular lesion (Table 3.3.4).

Table 3.3.4: Prevalence of subocclusal infundibular lesions identified by computed tomographic examination in each Triadan position in 100 maxillary cheek teeth.

TRIADAN POSITION	06	07	08	09	10	11	TOTAL
INFUNDIBULAR LESION	8	15	24	90	41	4	182
NO LESION	0	1	4	4	3	6	18
TOTAL	8	16	28	94	44	10	200
% WITH LESION	100	94	86	96	93	40	91

Relationship between subocclusal infundibular lesions and age group

There was no statistically significant difference between the presence of any infundibular lesion and age group ($p = 0.77$; Chi-Squared Test) (Table 3.3.5).

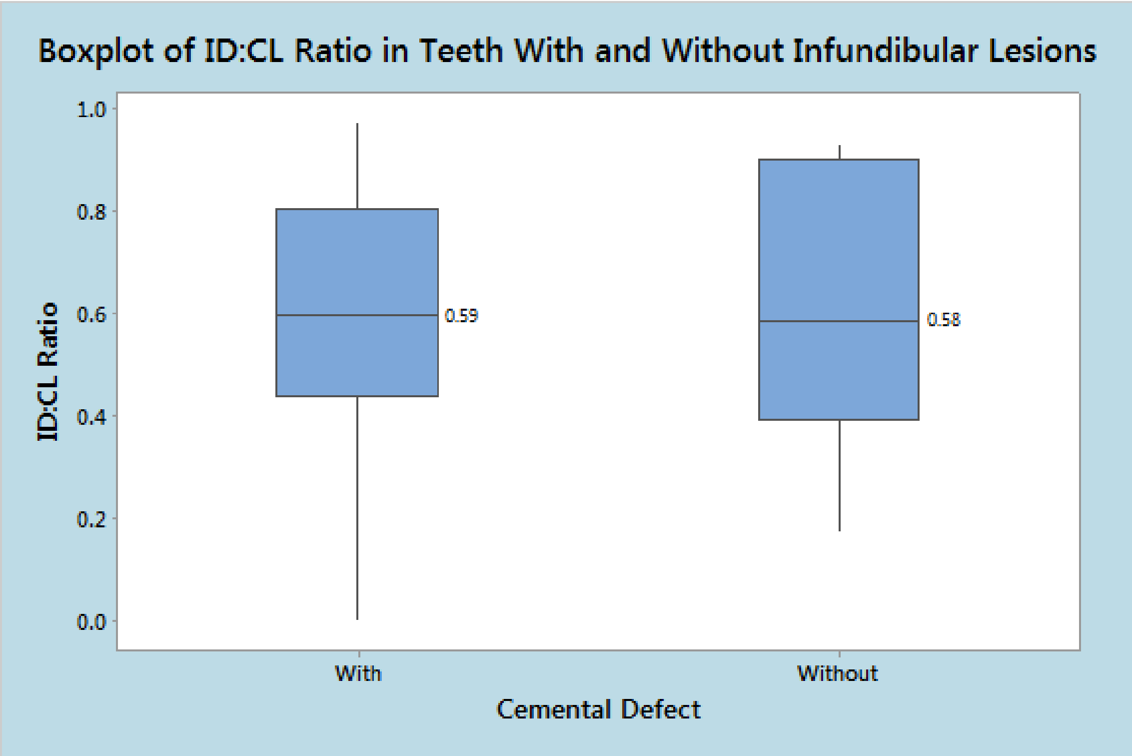
Table 3.3.5: Prevalence of subocclusal infundibular lesions identified by computed tomographic examination in 200 infundibulae classified by age group.

AGE (YEARS)	< 5	5-10	10-15	15-20	> 20	TOTAL
INFUNDIBULAR LESION	25	50	36	40	31	182
NO LESION	3	4	2	6	3	18
TOTAL	28	54	38	46	34	200
% WITH LESION	89	85	95	87	89	91

Relationship between subocclusal infundibular lesions and ID:CL ratio

The median ID:CL ratio of teeth with and without subocclusal infundibular lesions was very similar, with a slight difference in interquartile range (Figure 3.3.6).

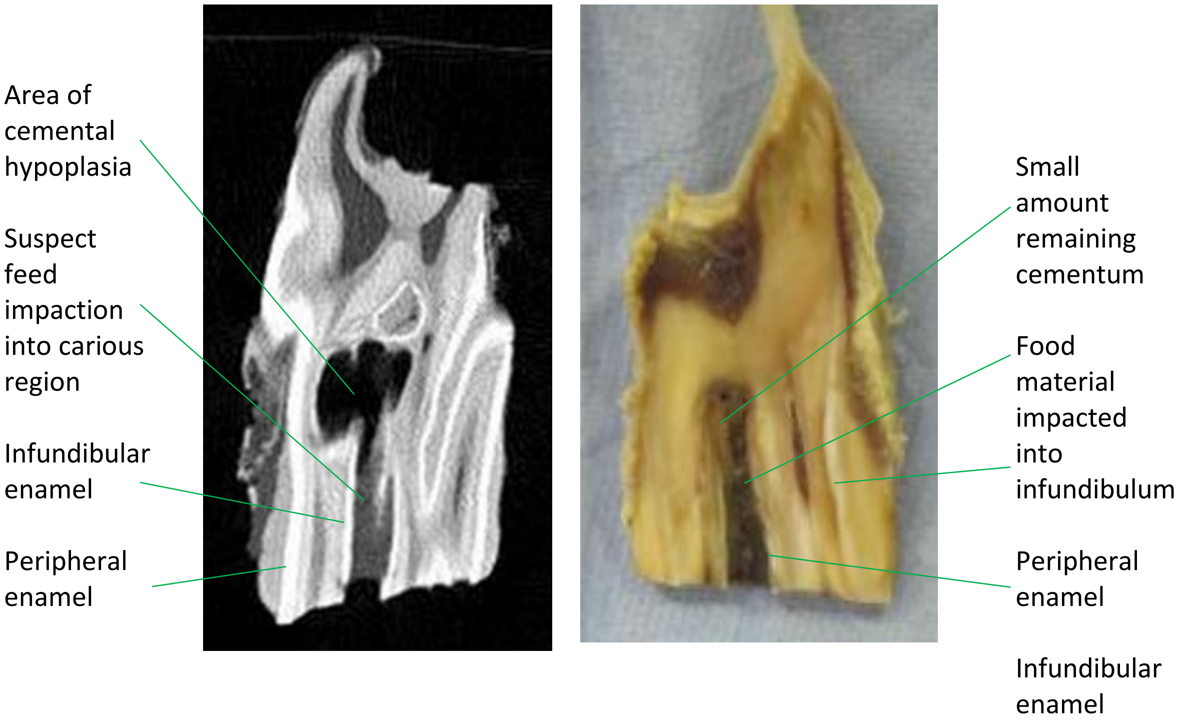
Figure 3.3.6: The boxplot depicts the median ID:CL ratio and interquartile range in 200 infundibulae with and without infundibular lesions.



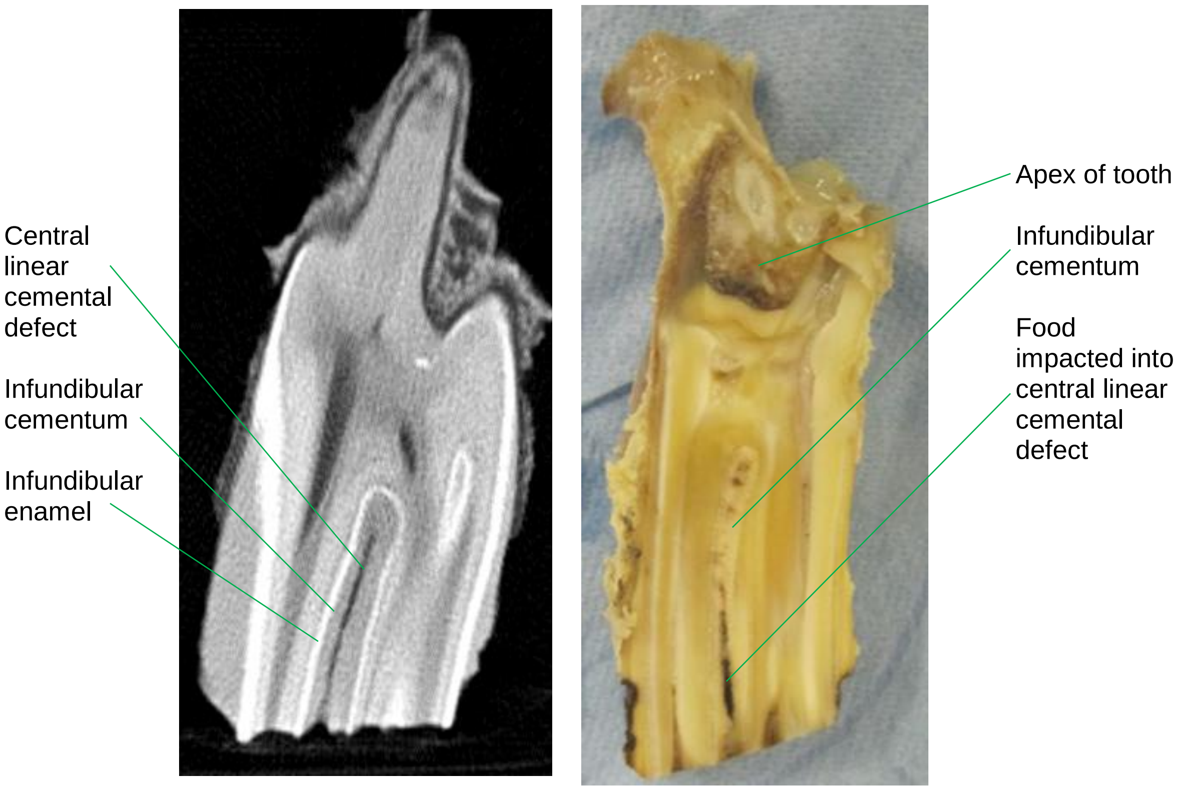
3.3.3 Morphological Characteristics of Infundibular Lesions on Computed Tomography

Subocclusal infundibular lesions were categorized as either a continuation of occlusal infundibular caries, central linear cemental defects, or apical cemental hypoplasia (Figure 3.3.7). The use of these categories of lesions was found to be satisfactory. Of 200 infundibulae examined, 167 (84%) had subocclusal lesions including 54 (27%) affected by caries extending from the occlusal surface, and 64 (32%) affected by central linear cemental defects only. Seven (3.5%) infundibulae were affected by apical cemental hypoplasia alone, but 42 infundibulae had both central linear defects and apical cemental hypoplasia. Only 18 (9%) infundibulae were completely unaffected by any infundibular lesion on CT scan, with another 15 infundibulae affected by occlusal surface caries alone (Figure 3.3.8).

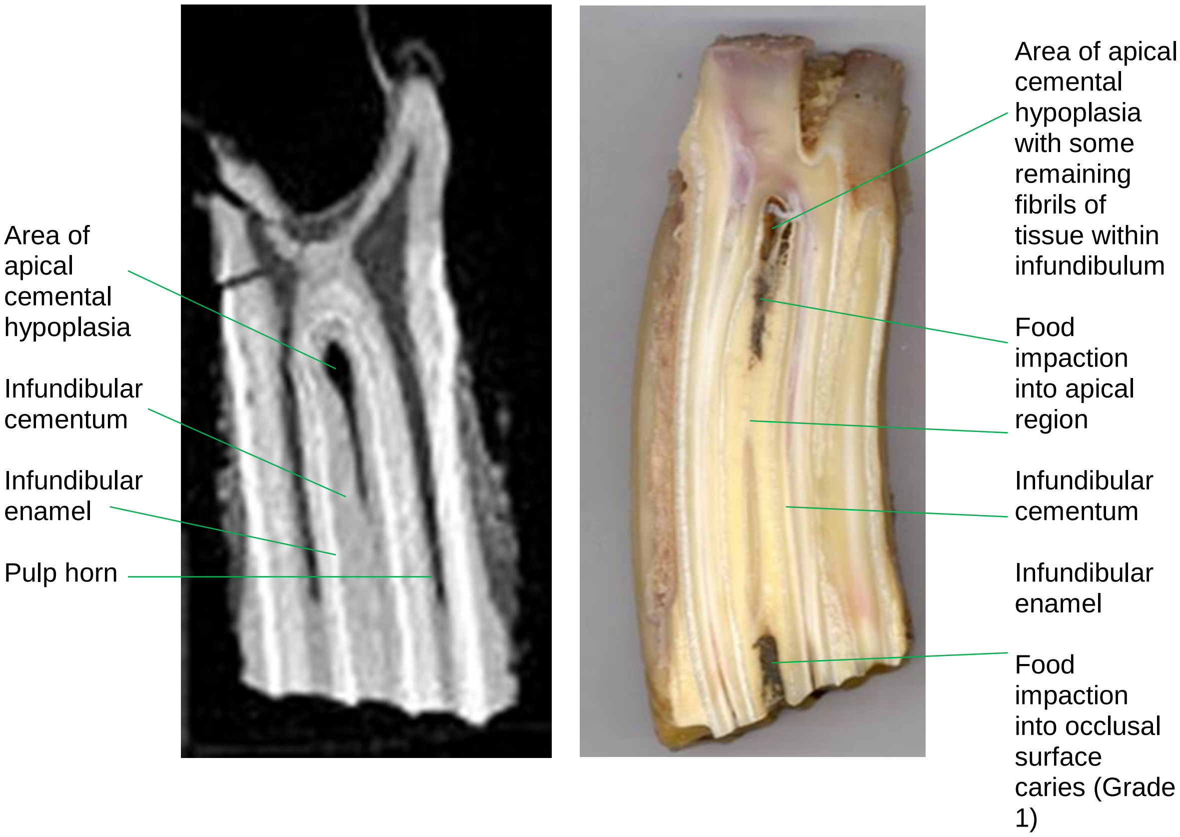
Figure 3.3.7: Computed tomographic scans (longitudinal sections) of maxillary cheek teeth and of similar gross anatomical longitudinal sections showing examples of different infundibular lesions.



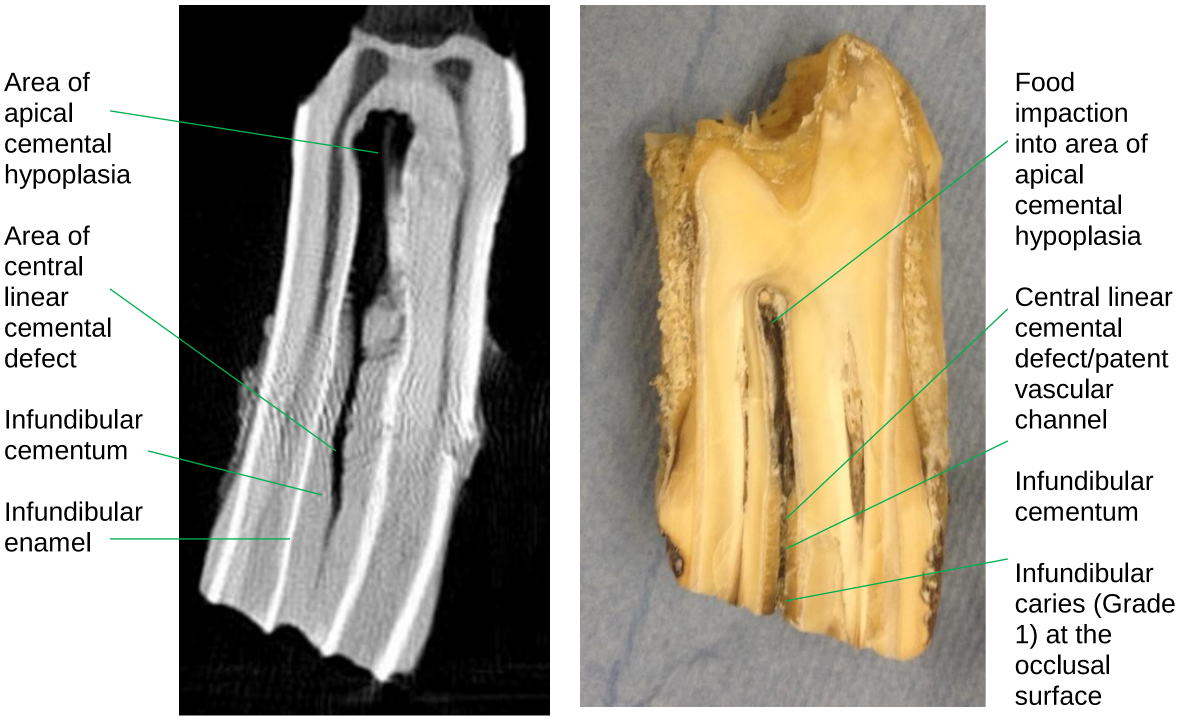
Extensive subocclusal infundibular caries continuous with occlusal surface caries



Central linear infundibular cemental defects

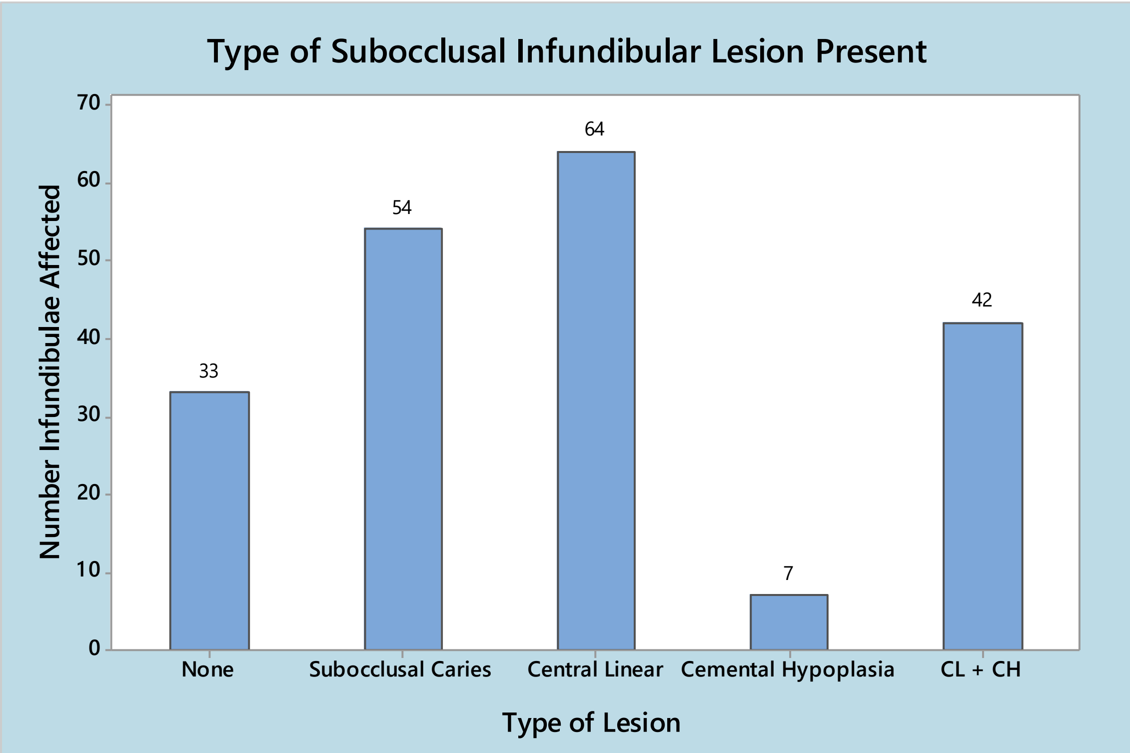


Apical cemental hypoplasia (with occlusal infundibular caries in the anatomical specimen)



Central Linear cemental defect and marked apical cemental hypoplasia (with food material in the central linear defect on gross section)

Figure 3.3.8: Distribution of sub-occlusal infundibular lesions imaged by computed tomography in 200 infundibulae. CL + CH indicates the infundibulum is affected by both a central linear (CL) cemental defect and apical cemental hypoplasia (CH).



Relationship between type of subocclusal lesion and infundibular position (rostral or caudal)

There was no statistically significant difference in the types of subocclusal infundibular lesions found between the rostral and caudal infundibulae ($p = 0.56$; Chi-Squared Test). The numbers of each defect found in the rostral and caudal infundibulae are displayed in Table 3.3.6 below.

Table 3.3.6: Numbers and types of infundibular lesions identified on computed tomography in the rostral and caudal infundibulae of 100 maxillary cheek teeth. CL = central linear defect; CH = cemental hypoplasia.

Subocclusal Infundibular Lesion	Rostral Infundibulum	Caudal Infundibulum	Total Infundibulae
None	13	20	33
Subocclusal Caries	31	23	54
Central Linear Defect (CL)	31	33	64
Cemental Hypoplasia (CH)	3	4	7
CL + CH	22	20	42
Total Infundibulae	100	100	200

Relationship between type of subocclusal infundibular lesion and Triadan position

The numbers and proportions of the various subocclusal infundibular lesions found in 200 infundibulae by computed tomography in the different Triadan positions are presented in Table 3.3.7 and also as a histogram Figure 3.3.9, respectively.

Due to the 06 and 11 Triadan teeth included in this analysis being unaffected by certain types of infundibular lesions, statistical analysis to examine the effect of Triadan position on the prevalence of the different types of cemental defects was not possible. However, the 09 teeth were found to be most frequently affected by subocclusal caries (37% prevalence), while the 06, 07, and 10 teeth were more commonly affected by central linear cemental defects (74%, 50%, and 43% prevalence respectively). Apical cemental hypoplasia was uncommonly found (Table 3.3.8). Most Triadan positions had a similar proportion (13-14%) of infundibulae without lesions, with the exception of the 06 position where all teeth had some type of lesion, and the 11 position (only 5 teeth included) in which 80% of infundibulae had no lesions.

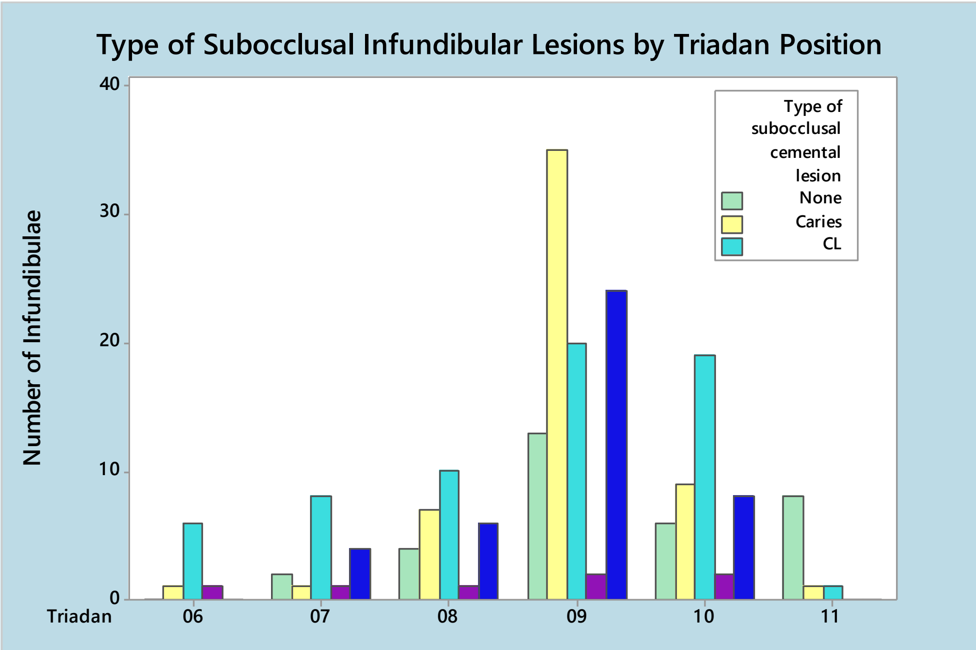
Table 3.3.7: Numbers of the different types of subocclusal infundibular lesions in the different Triadan positions identified in 100 maxillary cheek teeth by computed tomography.

TRIADAN POSITION	06	07	08	09	10	11	TOTAL
NONE	0	2	4	13	6	8	33
CARIES	1	1	7	35	9	1	54
CL	6	8	10	20	19	1	64
CH	1	1	1	2	2	0	7
CL + CH	0	4	6	24	8	0	42
TOTAL NUMBER	8	16	28	94	44	10	200

Table 3.3.8: Proportion of infundibulae in 100 maxillary cheek teeth affected by different types of subocclusal infundibular lesions identified by computed tomography in the different Triadan Positions. CL = central linear defect; CH = cemental hypoplasia.

TRIADAN POSITION	06	07	08	09	10	11	TOTAL
NONE	0%	13%	14%	14%	14%	80%	17%
CARIES	13%	6%	25%	37%	20%	10%	27%
CL	74%	50%	36%	21%	43%	10%	32%
CH	13%	6%	4%	2%	5%	0	4%
CL + CH	0	25%	21%	26%	18%	0	21%

Figure 3.3.9: Types of subocclusal infundibular lesions found on computed tomography in the different Triadan positions of 100 maxillary cheek teeth.



Relationship between type of subocclusal infundibular lesion and age group

There was a statistically significant difference between the type of subocclusal lesions present in the different age groups ($p = 0.001$; Kruskal-Wallis Test). Some age groups were unaffected by certain subocclusal cemental defects and the prevalence of cemental hypoplasia alone was low in the two younger age groups, but was more often seen in combination with a central linear defect (Table 3.3.9, Table 3.3.10, and Figure 3.3.10).

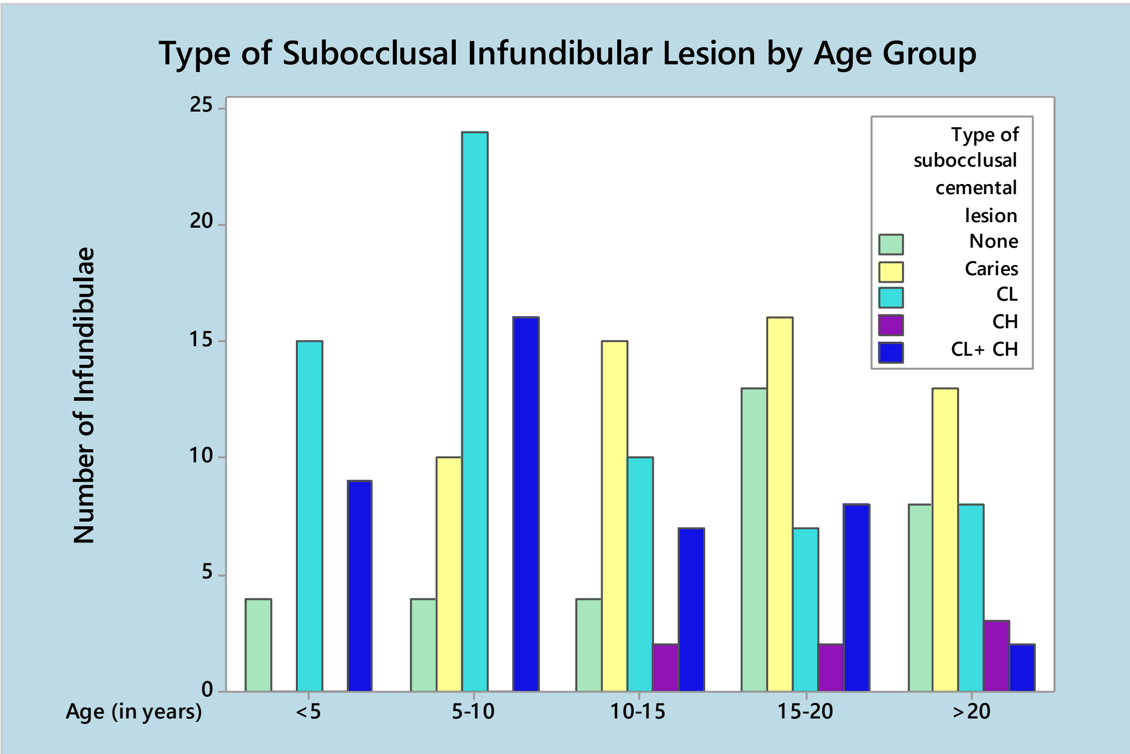
Table 3.3.9: Numbers of the different types of subocclusal infundibular lesions in 100 maxillary cheek teeth identified by computed tomography in the different age groups. CL = central linear defect; CH = cemental hypoplasia.

AGE (YEARS)	< 5	5-10	10-15	15-20	> 20	TOTAL
NONE	4	4	4	13	8	33
CARIES	0	10	15	16	13	54
CL	15	24	10	7	8	64
CH	0	0	2	2	3	7
CL + CH	9	16	7	8	2	42
TOTAL	28	54	38	46	34	200

Table 3.3.10: Proportion of infundibulae in 100 maxillary cheek teeth affected by different types of subocclusal infundibular lesions in different age groups identified by computed tomography. CL = central linear defect; CH = cemental hypoplasia.

AGE (YEARS)	< 5	5-10	10-15	15-20	> 20	TOTAL
NONE	14%	7%	11%	28%	24%	17%
CARIES	0	19%	39%	35%	38%	27%
CL	54%	44%	26%	15%	24%	32%
CH	0	0	5%	4%	9%	4%
CL + CH	32%	30%	18%	17%	6%	21%

Figure 3.3.10: Type of subocclusal infundibular lesions seen on computed tomography in the different age groups in 100 maxillary cheek teeth. CL = central linear defect; CH = cemental hypoplasia.



Relationship between occlusal and subocclusal infundibular lesions

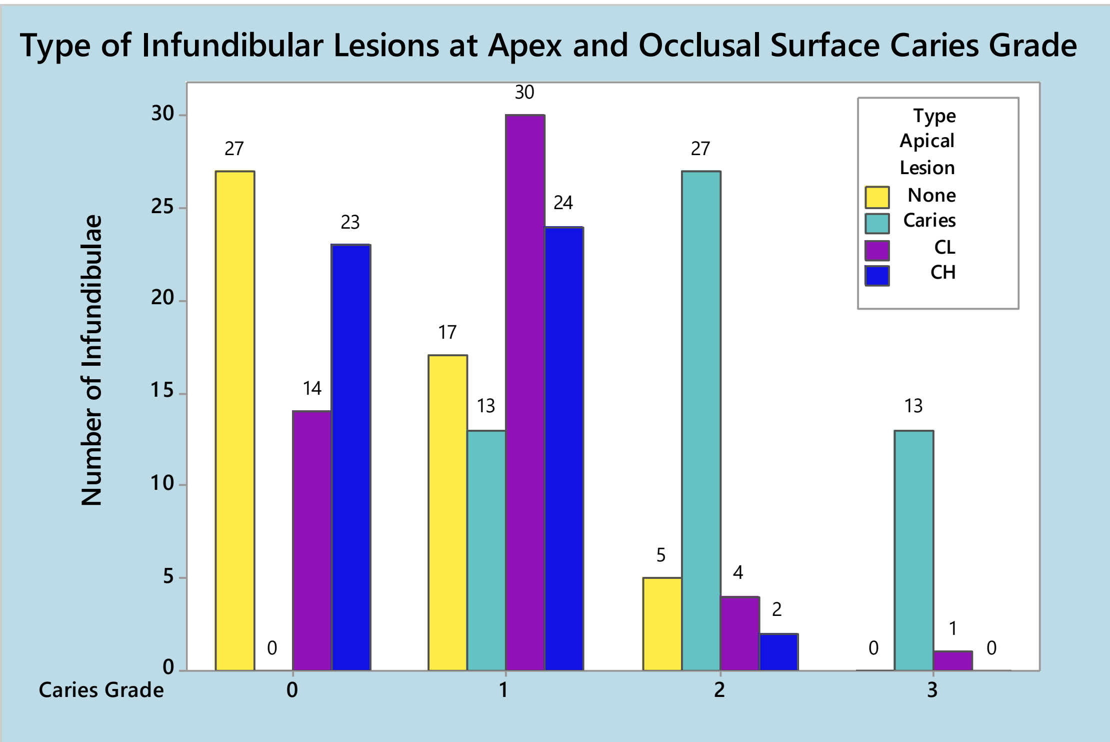
Teeth affected by occlusal infundibular caries were significantly more frequently affected by subocclusal cemental lesions than teeth without occlusal infundibular caries ($p = 0$; Chi-Squared Test) (Table 3.3.11).

Table 3.3.11: Prevalence of different types of subocclusal infundibular lesions as identified by computed tomography in 200 infundibulae with and without concurrent occlusal infundibular lesions.

Subocclusal Lesions	Occlusal Caries	No Caries	Total Infundibulae
None	14	19	33
Caries	54	0	54
CL	42	22	64
CH	2	5	7
CL + CH	24	18	42
Total	136	64	200

These data may be confounded by the fact that by default, teeth with subocclusal caries always have occlusal surface caries as well. Similarly, the association of subocclusal cemental defects with the grade of the occlusal surface caries was unable to be calculated as teeth affected by more severe (grade 2 and grade 3) occlusal infundibular caries are affected by caries at the apex of the infundibulum (71% and 93% respectively) but were rarely (between 0-2%) affected by central linear defects, apical cemental hypoplasia, or a combination of these developmental cemental defects at the apex of the infundibulum (Figure 3.3.11).

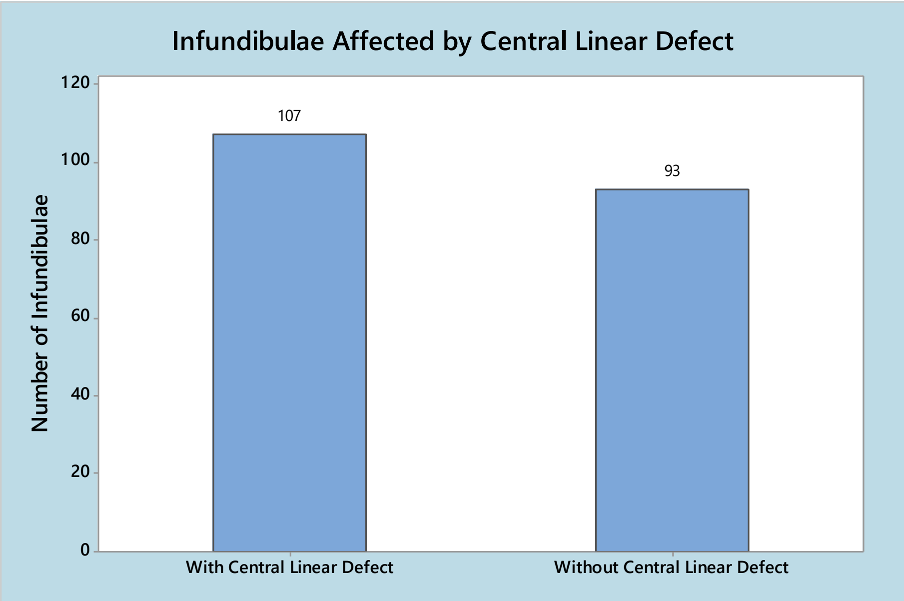
Figure 3.3.11: Types of apical infundibular lesions found by computed tomography in infundibulae with different grades of occlusal caries.



3.3.4 Central Linear Cemental Defects

Central linear cemental defects were identified by computed tomography in 107/200 infundibulae (54%). (Figure 3.3.12).

Figure 3.3.12: Numbers of infundibulae affected and unaffected by central linear cemental defects detected by computed tomography in 100 maxillary cheek teeth.



Relationship between central linear cemental defects and occlusal infundibular caries

There was a statistically significant difference in the presence of a central linear defect between teeth with visible occlusal infundibular caries (49% affected) and those without occlusal infundibular defects (64% affected) (Chi-Squared Test; $p = 0.04$) (Table 3.3.12).

Table 3.3.12: Numbers of central linear cemental defects in 100 maxillary cheek teeth detected by computed tomography in infundibulae with or without visible occlusal surface caries.

	No Central Linear Defect	Affected by Central Linear Defect	Number of Infundibulae
No Occlusal Caries	23	41	64
Occlusal Caries	70	66	136
Number of Infundibulae	93	107	200

There was also a statistically significant difference in the prevalence of central linear cemental defects between infundibulae with different grades of occlusal infundibular caries, with the infundibulae without visible occlusal lesions (Grade 0) and those with mild (Grade 1) occlusal caries more likely to be affected by central linear defects (Chi-Squared Test; p = 0) (Table 3.3.13) than those with higher grades of occlusal caries.

Table 3.3.13: Prevalence of central linear defects detected by computed tomography in 200 infundibulae classified by grade of occlusal caries.

CARIES GRADE	NUMBER OF INFUNDIBULAE	NO CENTRAL LINEAR DEFECT	CENTRAL LINEAR DEFECT PRESENT	% AFFECTED BY CENTRAL LINEAR DEFECT
GRADE 0	64	23	41	64%
GRADE 1	84	25	59	70%
GRADE 2	38	32	6	16%
GRADE 3	14	13	1	7%
TOTAL	200	93	107	54%

Relationship between central linear defects and infundibular position (rostral and caudal)

There was no statistically significant difference in the distribution of central linear defects between the rostral and caudal infundibulae (McNemar's Test; p = 1) (Table 3.3.14).

Table 3.3.14: Prevalence of central linear defects (CL) detected by computed tomography in the rostral and caudal infundibulae of 100 maxillary cheek teeth.

Infundibulum	Rostral With CL	Rostral Without CL	Total Infundibulae
Caudal With CL	42	11	53
Caudal Without CL	12	35	47
Total	54	46	100

Relationship between central linear defects and Triadan position

However, there was a statistically significant difference between Triadan positions affected by central linear defects (Chi-Square Test; p = 0.005), with the Triadan 11 position least likely to be affected by a central linear defect (10%), and the 07 and 06 positions most likely to be affected by a central linear defect (81% and 75% respectively) (Table 3.3.15).

Table 3.3.15: Prevalence of central linear defects (CL) detected by computed tomography in 200 infundibulae classified by Triadan position.

TRIADAN POSITION	06	07	08	09	10	11	TOTAL
CL DEFECT	6	13	16	44	27	1	107
NO CL DEFECT	2	3	12	50	17	9	93
TOTAL NUMBER	8	16	28	94	44	10	200
% WITH CL DEFECT	75	81	57	47	61	10	68

Relationship between central linear cemental defects and age group

There was also a statistically significant difference in the prevalence of central linear defects between age groups (Chi-Squared Test; p = 0), with younger horses more likely to be affected by central linear defects than older horses (Table 3.3.16).

Table 3.3.16: Prevalence of central linear (CL) defects detected by computed tomography in 200 infundibulae classified by age group.

AGE (YEARS)	< 5	5-10	10-15	15-20	> 20	TOTAL
CENTRAL LINEAR DEFECT	24	40	17	15	11	107
NO DEFECT	4	14	21	31	23	93
TOTAL NUMBER	28	54	38	46	34	200
% AFFECTED	86	74	45	33	32	54

3.3.5 Apical Cemental Hypoplasia (CH)

Apical cemental hypoplasia was present in 49/200 infundibulae (25%) (Figure 3.3.13). The proportion of infundibulae affected by central linear cemental defects and apical cemental hypoplasia is presented in Figure 3.3.14.

Figure 3.3.13: Numbers of infundibulae affected with and unaffected by apical cemental hypoplasia in 100 maxillary cheek teeth

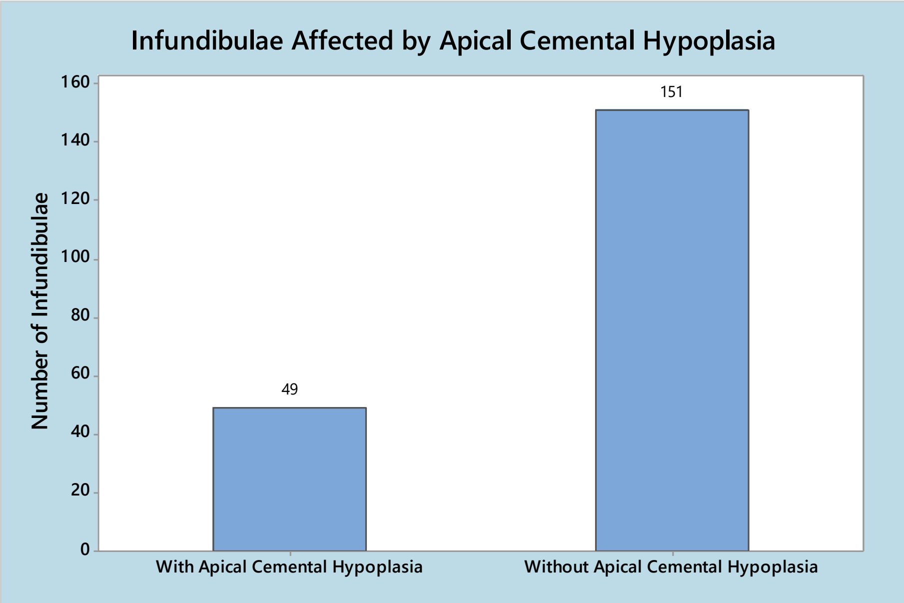
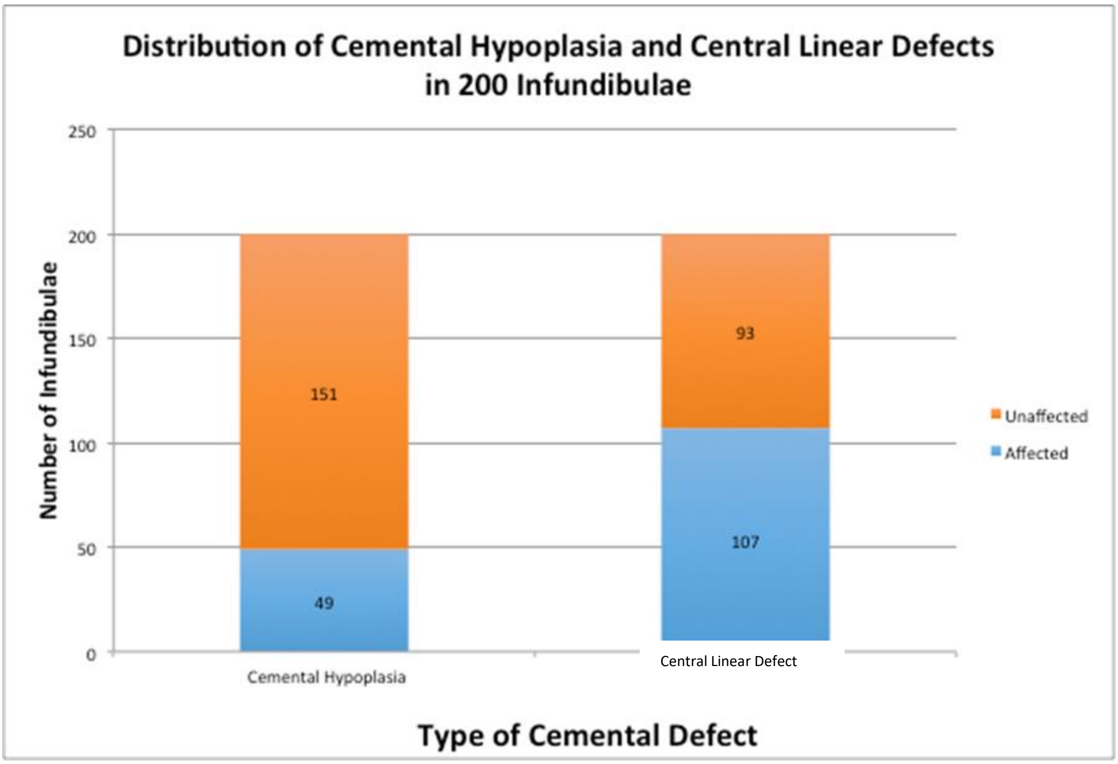


Figure 3.3.14: Distribution of cemental hypoplasia and central linear cemental defects (affected = blue; unaffected = orange) detected by computed tomography in 200 infundibulae.



Relationship between apical cemental hypoplasia and occlusal infundibular caries

There was a statistically significant difference in the presence of apical cemental hypoplasia between teeth with and without visible occlusal infundibular caries (Chi-Squared Test; $p = 0.01$). A large proportion (81%) of teeth with occlusal infundibular caries were unaffected by apical cemental hypoplasia. Teeth with apical cemental hypoplasia, however, were similarly affected by visible occlusal caries (53%; n = 26) and unaffected by visible occlusal caries (47%; n = 23) (Table 3.3.17).

Table 3.3.17: Prevalence of apical cemental hypoplasia detected by computed tomography in infundibulae affected with or without visible occlusal surface caries.

	No Apical Cemental Hypoplasia	Affected by Apical Cemental Hypoplasia	Total Infundibulae
No Caries	41	23	64
Caries	110	26	136
Total Infundibulae	151	49	200

There was also a statistically significant difference in the prevalence of apical cemental hypoplasia between infundibulae affected by different grades of occlusal caries, with infundibulae without visible occlusal surface lesions(Grade 0) and those with mild (Grade 1) caries more likely to be affected by apical cemental hypoplasia than infundibulae with more severe grades of occlusal caries (Chi-Squared Test; $p = 0.001$). Infundibulae with more severe occlusal caries (Grade 2 and Grade 3) were rarely affected by apical cemental hypoplasia (Table 3.3.18).

Table 3.3.18: Prevalence of apical cemental hypoplasia detected by computed tomography in 200 infundibulae classified by grade of occlusal infundibular caries present.

CARIES GRADE	AFFECTED BY CEMENTAL HYPOPLASIA	NO CEMENTAL HYPOPLASIA	TOTAL NUMBER OF INFUNDIBULAE
GRADE 0	23	41	64
GRADE 1	24	60	84
GRADE 2	2	36	38
GRADE 3	0	14	14
TOTAL	49	151	200

Relationship between apical cemental hypoplasia and Triadan position

There was no statistically significant difference in the prevalence of apical cemental hypoplasia (CH defect) between the different Triadan positions (Chi-Squared Test; $p = 0.44$). The 07 cheek teeth were most commonly affected by apical CH (31%) while the 11 teeth were unaffected by apical CH in these samples (Table 3.3.19).

Table 3.3.19: Prevalence of apical cemental hypoplasia (CH) detected by computed tomography in 200 infundibulae classified by Triadan position.

TRIADAN POSITION	06	07	08	09	10	11	TOTAL
CH DEFECT	1	5	7	26	10	0	49
NO CH DEFECT	7	11	21	68	34	10	151
TOTAL	8	16	28	94	44	10	200
% WITH CH DEFECT	13	31	25	28	23	0	25

Relationship between apical cemental hypoplasia and infundibular position (rostral or caudal)

There was also no significant difference between the prevalence of apical cemental hypoplasia between paired rostral and caudal infundibulae (McNemar's Test; $p = 1$). In 63% of teeth, both infundibulae were unaffected by apical cemental hypoplasia (Table 3.3.20).

Table 3.3.20: Comparison of prevalence of apical cemental hypoplasia detected by computed tomography in paired rostral and caudal infundibulae in 100 maxillary cheek teeth.

Infundibulum	Rostral With CH	Rostral Without CH	Total
Caudal With CH	12	12	24
Caudal Without CH	13	63	76
Total	25	75	100

Relationship between apical cemental hypoplasia and age group

There was also no significant difference in the prevalence of cemental hypoplasia between the different age groups (Chi-Squared Test; $p = 0.46$). However, there was a steady decline in the proportion of teeth affected by cemental hypoplasia from 32% in horses <5 years old to 15% in horses >20 years old (Table 3.3.21).

Table 3.3.21: Prevalence of apical cemental hypoplasia detected by computed tomography in 200 infundibulae classified by age group.

AGE (YEARS)	< 5	5-10	10-15	15-20	> 20	TOTAL
CEMENTAL HYPOPLASIA (CH)	9	16	9	10	5	49
NO CH	19	38	29	36	29	151
TOTAL	28	54	38	46	34	200
% WITH CH	32	30	24	22	15	25

3.3.6 Combined Apical Infundibular Lesions

The apical aspect of the infundibulum could be affected by all of the aforementioned lesions including developmental apical cemental hypoplasia and central linear cemental defects along with acquired subocclusal caries. A total of 151/200 (75.5%) infundibulae, involving 87/100 (87%) teeth, were affected by any type of apical infundibular lesion. Only 31/200 infundibulae (16%) were affected by a cemental defect that did not reach the apical aspect of the infundibulum. Teeth affected by occlusal surface caries were very likely (84%; 114/136) to have an apical cemental defect as well.

Some type of apical cemental lesion was present in 82% of rostral infundibulae and 69% of caudal infundibulae (Table 3.3.22).

Table 3.3.22: Numbers of rostral and caudal infundibulae and teeth affected and unaffected with any type of apical infundibular lesion detected by computed tomography in 100 maxillary cheek teeth.

Infundibulum	Rostral Affected	Rostral Unaffected	Number of Teeth
Caudal Affected	64	5	69
Caudal Unaffected	18	13	31
Total	82	18	100

The prevalence of apical cemental lesions in the different age groups and Triadan positions is shown in Table 3.3.23 and 3.3.24 respectively.

Table 3.3.23: Prevalence of apical cemental defects detected by computed tomography in 200 infundibulae classified by age group.

AGE (YEARS)	< 5	5-10	10-15	15-20	> 20	TOTAL
APICAL LESION	20	41	33	31	26	151
NO LESION	8	13	5	15	8	49
TOTAL NUMBER	28	54	38	46	34	200
% WITH APICAL LESION	71	76	87	67	76	76

Table 3.3.24: Prevalence of apical cemental defects detected by computed tomography in 200 infundibulae classified by Triadan position.

TRIADAN	06	07	08	09	10	11	TOTAL
APICAL LESION	5	14	20	82	29	1	151
NO LESION	3	2	8	12	15	9	49
TOTAL	8	16	28	94	44	10	200
% WITH APICAL LESION	63	88	71	87	66	10	76

3.3.7 Occult Subocclusal Infundibular Lesions in Teeth Without Visible Occlusal Lesions

Of the 18 teeth with visible occlusal surface infundibular lesions, 14 were found to have occult subocclusal infundibular defects on CT scan (78%). Both infundibulae were affected in 13/14 teeth (93%), with only the caudal infundibulum affected in 1/14 teeth (7%). Four teeth without visible occlusal surface lesions were also completely unaffected by an occult subocclusal infundibular cemental lesion (4/18; 22%).

Relationship between occult subocclusal infundibular lesions and Triadan position

Due to the high prevalence of occult infundibular lesions coupled with the absence of such lesions in some Triadan positions (07 and 11) (Table 3.3.25), statistical analysis to examine for differences in the prevalence of lesions between Triadan positions was not possible. However, both teeth obtained from the Triadan 11 position were completely unaffected by any infundibular cemental lesion.

Table 3.3.25: Prevalence of occult subocclusal infundibular lesions identified by computed tomographic examination in each Triadan position in 18 maxillary cheek teeth without visible occlusal surface lesions.

TRIADAN POSITION	06	07	08	09	10	11	TOTAL
INFUNDIBULAR LESION(S)	2	0	3	5	5	0	14
NO LESIONS	0	0	1	1	0	2	4
TOTAL	2	0	4	6	5	2	18
% AFFECTED	100	0	75	83	100	0	78

Relationship between occult subocclusal infundibular lesions and age group

Of the 18 teeth found to be without occlusal surface lesions, 8 were obtained from horses between 5-10 years in age. Of these, 6/8 (75%) had an occult subocclusal infundibular cemental defects. Due to the high prevalence of occult infundibular lesions, statistical analysis to examine for differences in the prevalence of occult lesions between age groups was not possible. Data regarding the prevalence of occult subocclusal infundibular cemental lesions in each age group is presented in Table 3.3.26.

Table 3.3.26: Prevalence of occult subocclusal infundibular lesions identified by computed tomographic examination in 18 teeth classified by age group.

AGE (YEARS)	< 5	5-10	10-15	15-20	> 20	TOTAL
INFUNDIBULAR LESION(S)	4	6	1	1	2	14
NO LESIONS	1	2	0	1	0	4
TOTAL	5	8	1	2	2	18
% WITH LESION	80	75	100	50	100	78

3.4 MicroCT and Histopathological findings

MicroCT was performed on 8 teeth of different ages as earlier described. Data collected on each tooth by visual examination of occlusal surface and by standard CT scans prior to microCT examination is shown in Table 3.4.1.

Table 3.4.1: Data on 8 teeth obtained by visual occlusal examination and standard CT evaluation.

Tooth	Triadan	Age Group (years)	Occlusal Caries Grade Rostral infundibulum	Occlusal Caries Grade Caudal infundibulum	Crown Length (cm)	Rostral Infundibulum Length (cm)	Caudal Infundibulum Length (cm)
A	108	< 5	0	1	7.04	5.46	6.00
B	109	< 5	1	1	7.91	6.54	6.92
C	109	5-10	0	1	5.67	4.38	3.93
D	109	5-10	1	0	5.95	4.64	5.09
E	209	10-15	1	1	6.57	3.00	3.38
F	109	15-20	3	3	5.4	1.95	2.46
G	109	15-20	1	1	5.18	3.41	2.37
H	210	> 20	1	0	5.59	2.34	2.17

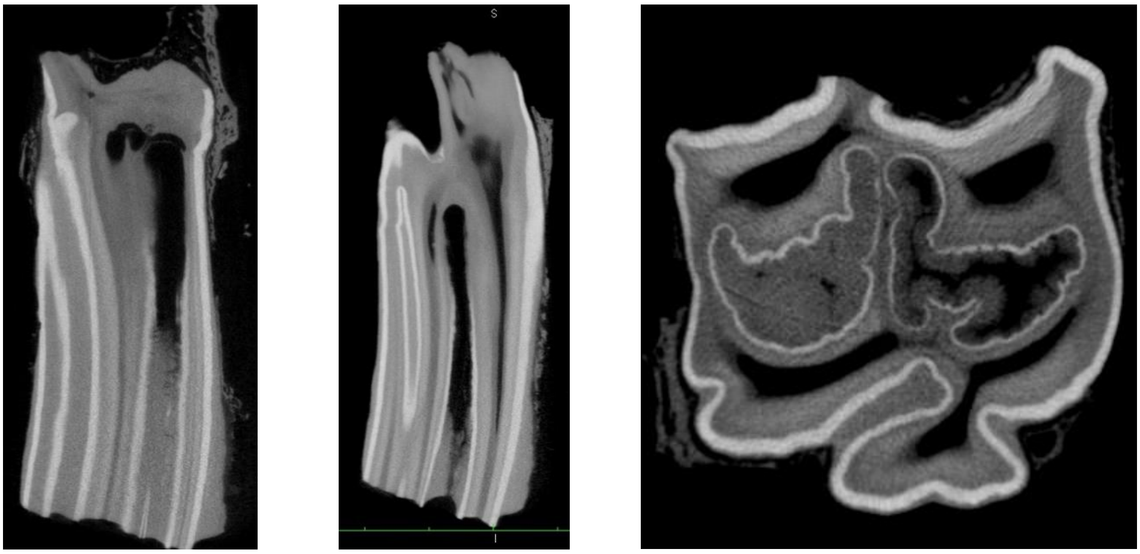
Lesions were identified in 15/16 infundibulae of these 8 teeth on standard CT examination. The types and locations of these lesions are presented in Table 3.4.2.

Table 3.4.2: Types and location of infundibular lesions detected on standard CT imaging in 8 maxillary cheek teeth.

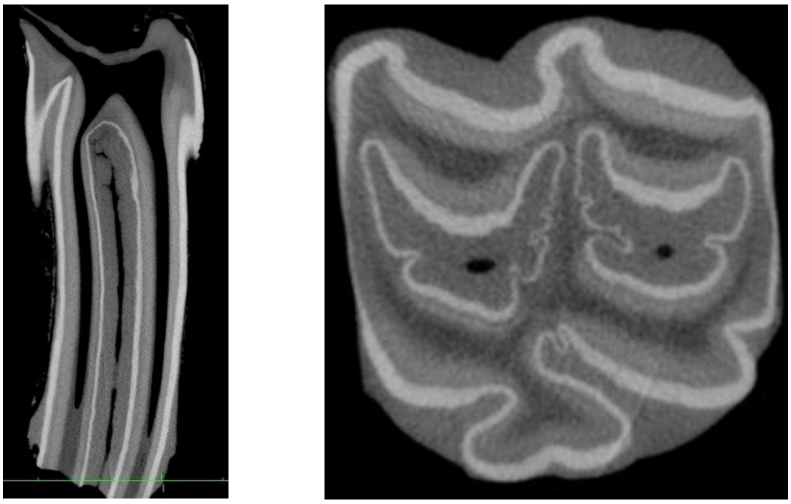
Tooth	Infundibulum	Occlusal Surface Caries Grade	Subocclusal Cemental Defects
A	Rostral	0	Central linear defect Apical cemental hypoplasia
	Caudal	1	Central linear defect
B	Rostral	1	Central linear defect
	Caudal	1	None – caries confined to occlusal surface only
C	Rostral	0	Central linear defect Apical cemental hypoplasia
	Caudal	1	Central linear defect Apical cemental hypoplasia
D	Rostral	1	Central linear defect Apical cemental hypoplasia
	Caudal	0	Central linear defect Apical cemental hypoplasia
E	Rostral	1	Central linear defect Apical cemental hypoplasia
	Caudal	1	Apical cemental hypoplasia
F	Rostral	3	Caries
	Caudal	3	Caries
G	Rostral	1	Central linear defect
	Caudal	1	Central linear defect
H	Rostral	1	Central linear defect Apical cemental hypoplasia
	Caudal	0	None

All infundibulae, except one, examined by standard CT or on occlusal surface examination were affected by at least one type of infundibular lesion (caries, central linear defect, or apical cemental hypoplasia). MicroCT scan reconstructions in both the longitudinal and transverse planes of each of these 8 teeth confirmed the presence of the infundibular lesions imaged on conventional CT in all samples. The microCT images however allowed much more detailed evaluation of the infundibular structure of each tooth in comparison to the standard CT images because the much thinner imaging slices allow for less volume averaging between slices of the imaged structure (Figure 3.4.1).

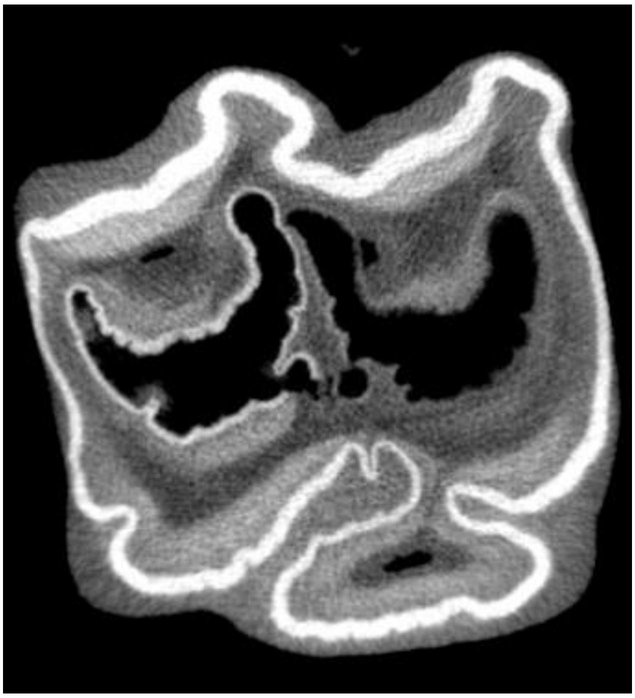
Figure 3.4.1: MicroCT images of various infundibular cemental filling defects (Images courtesy Dr Carsten Staszyk, Justus-Liebig-Universität Gießen, Gießen, Hesse, Germany)



Two longitudinal and one transverse microCT of maxillary cheek teeth affected by rostral infundibular apical cemental hypoplasia; a small, connecting central linear defect is also present beneath the occlusal surface in the central image



Examples of longitudinal and transverse microCT images of cheek teeth with central linear cemental defects of both infundibulae, that are small in the transverse image

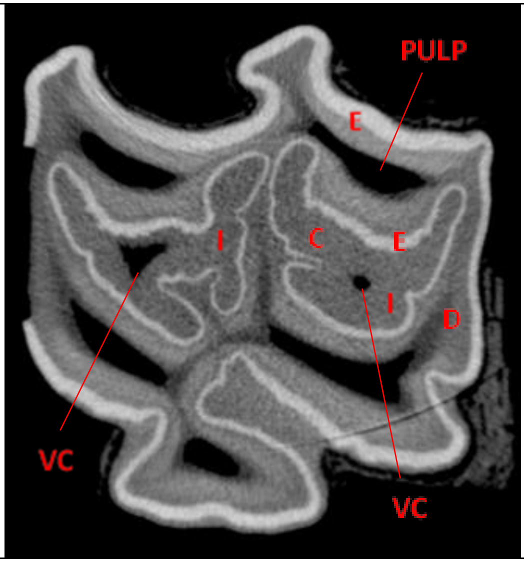
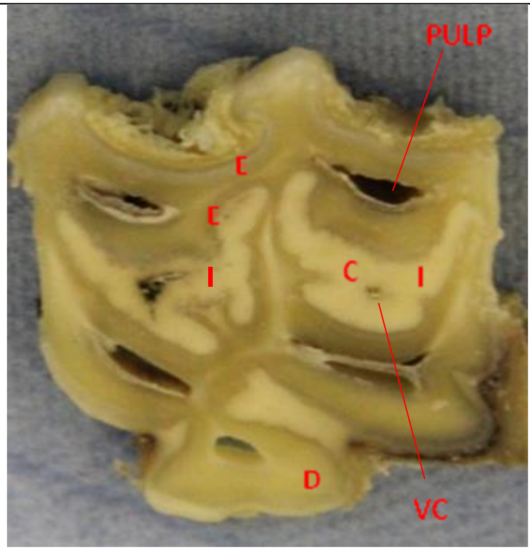
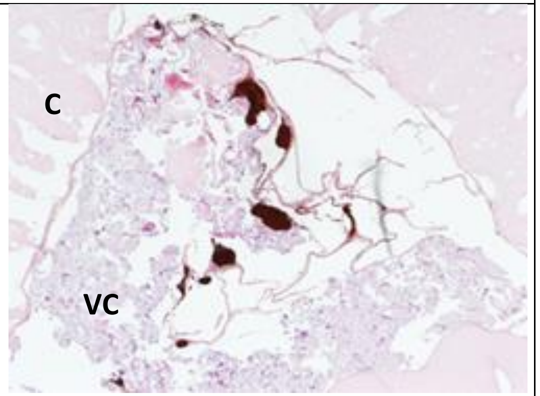


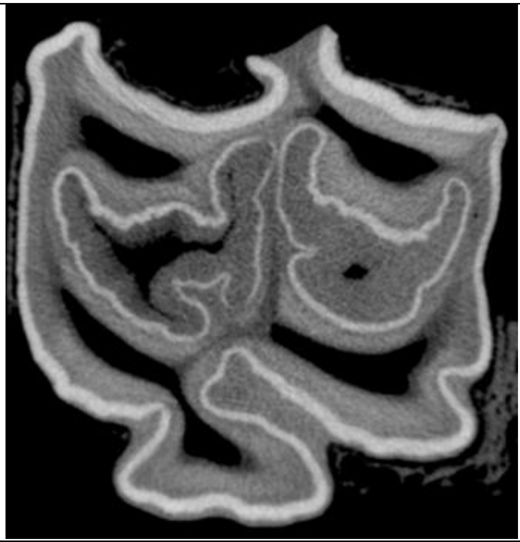
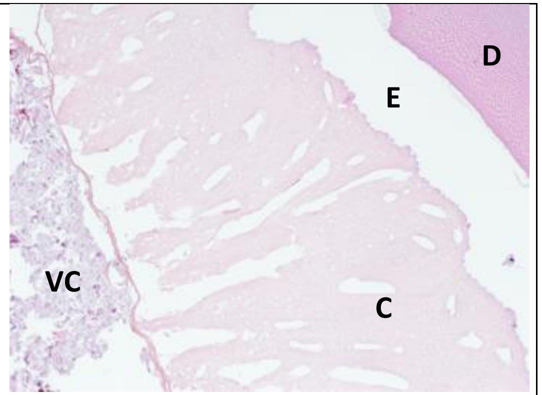
Transverse microCT image of Grade 2 (also involves enamel) caudal infundibular caries (left -rostral infundibulum) and Grade 3 (also involves dentine) rostral infundibular caries (right - caudal infundibulum)

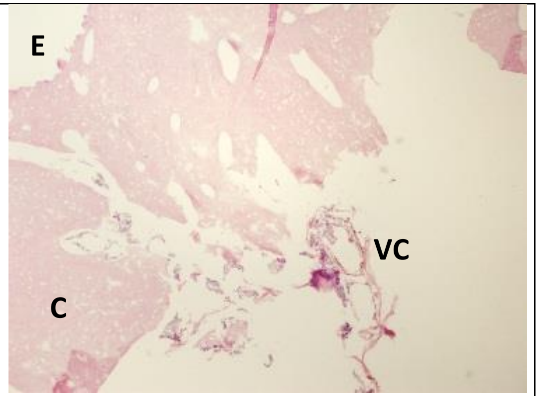
Transverse microCT image of combined central linear defect and apical cemental hypoplasia; note the close apposition of the infundibulum and pulp horn at a site of thin infundibular enamel (green line)

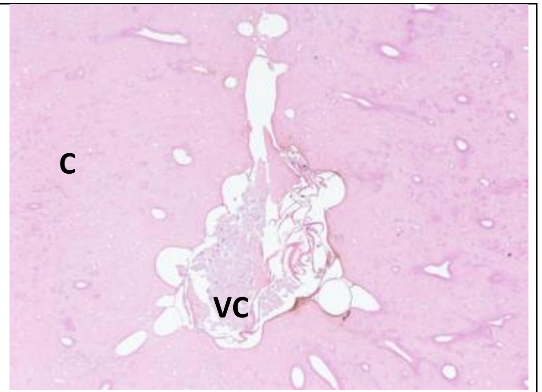
Following MicroCT imaging, the 8 teeth were transversely sectioned at areas of interest identified on microCT. Gross examination of these sections showed the infundibulae to be variably filled with cementum of different appearances (normal cream-coloured cementum; discoloured and/or porous cementum; or completely devoid of cementum) as depicted in Figure 3.4.2 along with the corresponding H&E stained histological sections. The included sections and graphics are examples used to illustrate the pathology that may be found in the infundibulae of each age group examined. More complete descriptions of the infundibular cemental histology are presented in the 'Histological Section' column of Figure 3.4.2 and in Table 3.4.3.

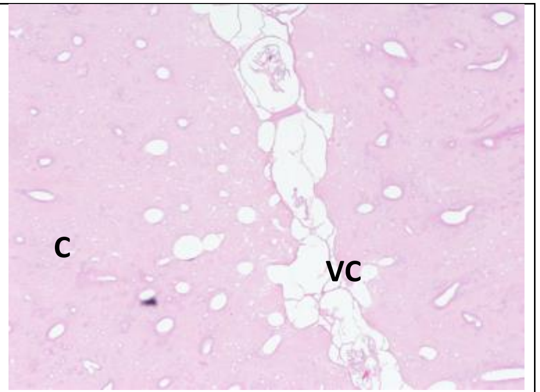
Figure 3.4.2: Transverse microCT images (Images courtesy Dr Carsten Staszyk, Justus-Liebig-Universität Gießen, Gießen, Hesse, Germany), gross, and histological sections of the selected samples of 8 maxillary cheek teeth. The rostral infundibulum is the left of each microCT and gross section image. In histological sections: I = Infundibulum; C = Cementum; VC = Central vascular channel; E = Enamel (decalcified); D = Dentine

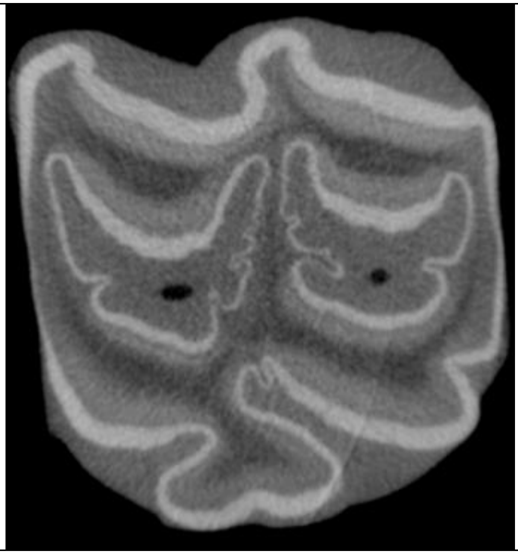
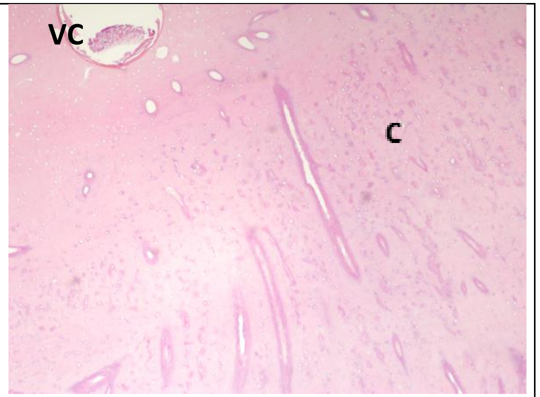
Tooth	Section Depth	MicroCT Image	Gross Transverse Section	Histological Section
A	30 mm			 Rostral Infundibulum Central Vascular Channel (4x Magnification)
		Rostral Infundibulum: Wide central linear defect beginning to branch towards infoldings - Diameter: 1.63 mm Caudal Infundibulum: Small central linear defect - Diameter: 0.69 mm	Rostral Infundibulum: localised area of porous, discoloured cementum surrounds the central vascular channel extending towards infoldings Caudal Infundibulum: Small area of discoloured cementum around the central vascular channel	• Fragments of plant material and degraded cementum within the central vascular channel • Brown material could be plant material; Prussian Blue stain confirmed no haemosiderin present • Many lateral vascular branches stemming from central vascular channel

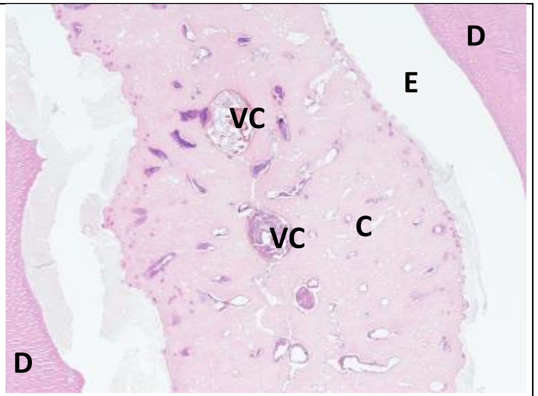
	40 mm			 Rostral Infundibulum Palatorostral Border (4x Magnification)
		Rostral Infundibulum: Area of cemental hypoplasia branching circumferentially from the central infundibulum into 3 infoldings - Diameter: 1.94 mm Caudal infundibulum: central linear defect - Diameter: 1.01 mm	Rostral Infundibulum: Discoloured cementum present in the central region of infundibular cementum and extending peripherally Caudal Infundibulum: Small amount discoloured cementum in central infundibulum	• Fragments of plant material and eroded cementum within the central vascular channel • Many wide side branches off the central vascular channel into cementum appear to be empty and gives the cementum a porous appearance

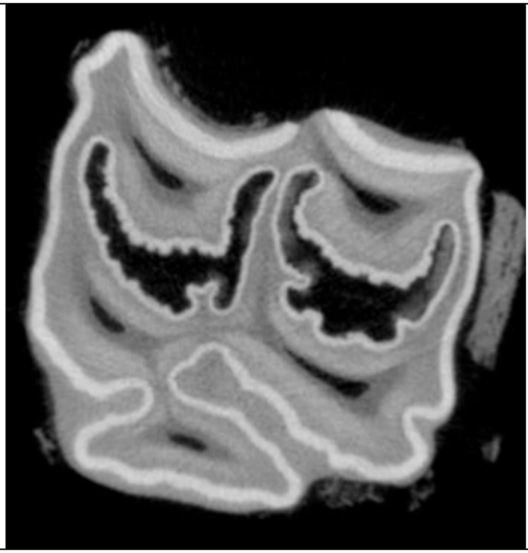
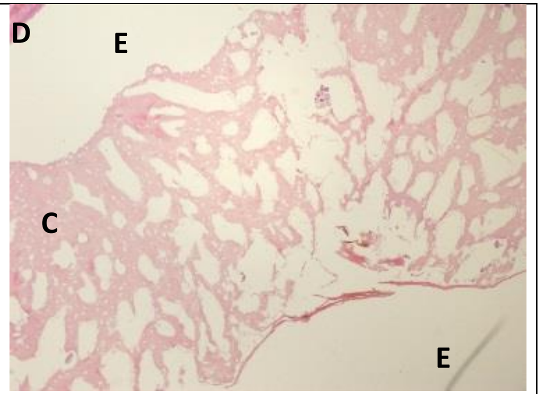
	55 mm			 Caudal Infundibulum Palatal Border (4x Magnification)
		Rostral Infundibulum: Only a small aspect of apex and of the caudal buccal infolding remain with cemental hypoplasia/absence in both Caudal Infundibulum: Small irregular area of cemental hypoplasia centrally and extending into the rostral buccal infolding	Rostral Infundibulum: Only enamel remains at apical aspect of infundibulum and a small area of discoloured cementum lies within the caudal buccal infolding Caudal Infundibulum: An irregular linear region of discoloured cementum centrally in infundibulum courses into the rostral buccal infolding	• Extensive,wide horizontal vascular branches throughout cementum with some containing plant material • Hypoplastic region surrounding the central vascular channel contains a small amount of plant and cellular debris

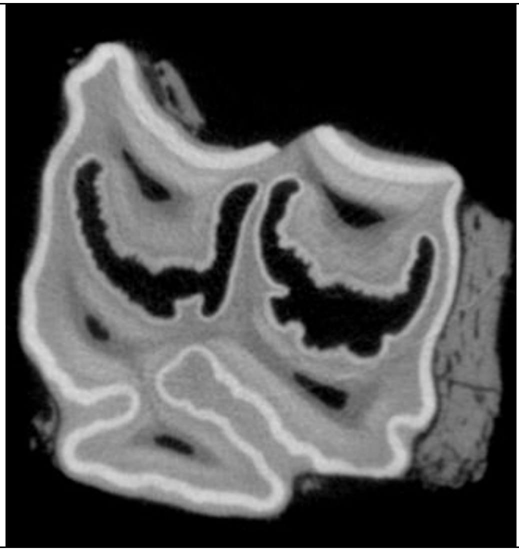
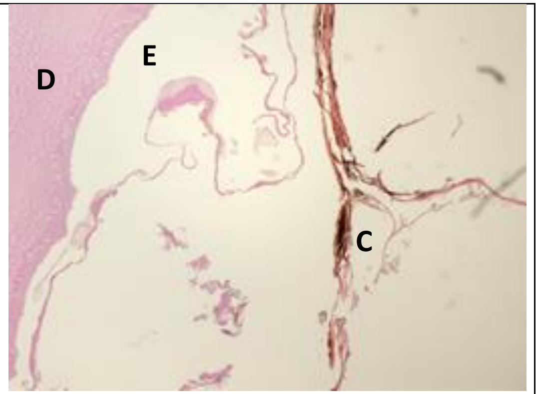
B	20 mm			 Caudal Infundibulum Central Vascular Channel (4x Magnification)
		Rostral Infundibulum: A central linear defect is present in the central vascular channel - Diameter: 1.12 mm Caudal Infundibulum: The central vascular channel (not centrally positioned here) is within normal limits - Diameter: 0.29 mm	Rostral Infundibulum: Discoloured cementum present in the region of the central vascular channel Caudal Infundibulum: Discoloured cementum present in the region of the central vascular channel	• Central vascular channel contains pale cellular debris but no evidence of plant material • Long horizontal, narow diameter lateral branches originate from the central vascular channel (not fully imaged)

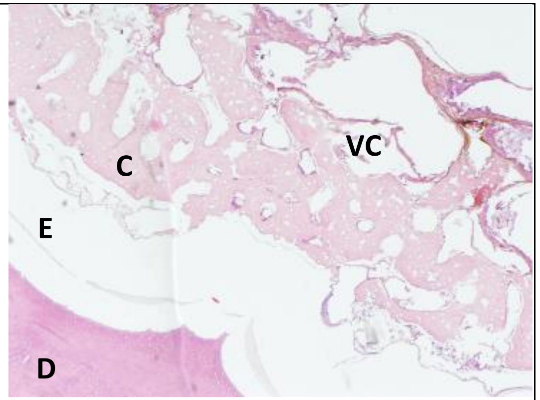
	50 mm			 Rostral Infundibulum Vascular Channel Defect (4x Magnification)
		Rostral Infundibulum: A thin linear area of cemental hypoplasia runs sagitally in central aspect of infundibulum Caudal infundibulum: Very small hypointense area present in region of central vascular channel; within normal limits	Rostral Infundibulum: Central linear area of discoloured cementum is more marked caudallly and extends to the caudal buccal infolding Caudal Infundibulum: Very small area of discoloured cementum in the region of the central vascular channel	• Central vascular channel has extensive horizontal branching • Fragmented pieces of cementum present centrally in the vascular channel

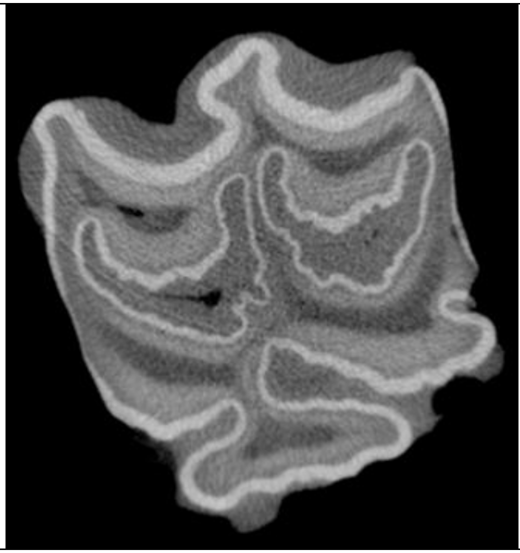
C	1-2 mm			 Caudal Infundibulum Central Vascular Channel and Lateral Branches (4x Magnification)
		Rostral Infundibulum: Central vascular channel - Diameter: 0.74 mm; within normal limits Caudal Infundibulum: Central vascular channel - Diameter: 0.51 mm; within normal limits	Rostral Infundibulum: Small rim of discoloured cementum in the region of the central vascular channel Caudal Infundibulum: Small rim of discoloured cementum in the region of the central vascular channel	• Fragments of plant material and degraded cementum within the central vascular channel • Small amount of brown material in central vascular channel; Prussian Blue stain confirmed no haemosiderin present

	15 mm			 Rostral Infundibulum Branched Vascular Channels (2x Magnification)
		Rostral Infundibulum: Cemental hypoplasia surrounding the central vascular channel region and extending to the buccal edge of the infundibulum Caudal Infundibulum: Thin linear central defect - Diameter: 1.04 mm	Rostral Infundibulum: A linear pattern of pale cementum lies around the central vascular channel Caudal Infundibulum: Pale cementum in the region of the central vascular channel	• Two central vascular channels are apparent and filled with plant material and pale pink, cellular material • Marked lateral branches either empty or contain cellular material without evidence of food impaction

D	30 mm			 Caudal Infundibulum Buccal Infolding (2x Magnification)
		Rostral Infundibulum: Complete cemental hypoplasia; appears to be some hypointensity of the internal enamel rim in the buccal region - Width of central area: 3.31 mm Caudal Infundibulum: Marked central cemental hypoplasia; some hypointense cementum remaining in buccal infoldings - Width of central area: 3.56 mm	Rostral Infundibulum: Complete cemental hypoplasia with some discolouration of surrounding enamel Caudal Infundibulum: Complete central cemental hypoplasia with some enamel discoloration. Cementum of apparently normal appearance remains in buccal infoldings	• Cementum has a marked moth-eaten appearance, not apparently due to blood vessel sites • Small basophilic pockets in cement appear to contain minerals • Darker pink strands (at bottom of cementum) appear to be collagen

	40 mm			 Caudal Infundibulum Amelocemental Junction (4x Magnification)
		Rostral Infundibulum: Complete cemental hypoplasia and hypointensity of surrounding enamel Caudal Infundibulum: Complete cemental hypoplasia and hypointensity loss of surrounding enamel	Rostral Infundibulum: Almost complete cemental hypoplasia (except in buccal infoldings) and discolouration of surrounding enamel Caudal Infundibulum: Almost complete central cemental hypoplasia (except infoldings) and discolouration of surrounding enamel	• Infundibulum contains strands of cementum that has become fibrillated as it degenerated • Cemental remnants appear to be peeling off the amelocemental junction

E	15 mm			 Rostral Infundibulum Central Palatal Margin (2x Magnification)
		Rostral Infundibulum: Thin linear area of cemental hypoplasia running along buccal margin of this narrow infundibulum; destruction of surrounding enamel especially at caudobuccal infolding Caudal Infundibulum: Large, poorly defined central linear defect - Diameter: 1.76 mm	Rostral Infundibulum: Discoloured, porous cementum within most of the infundibulum, most pronounced on buccal margin; discolouration and loss of defintion of surrounding enamel Caudal Infundibulum: Discoloured,porous cementum surrounds the central vascular channel with discolouration of central aspect of buccal enamel	• Cementum peeling and forming fibrils in region of central vascular channel • Remaining cementum has moth-eaten appearance • Some basophilic stipling in cementum • Brown material in central vascular channel either plant or haemosiderin ○ Prussian Blue stain confirmed no haemosiderin present

H	10 mm			 Rostral Infundibulum Buccal Infolding (2x Magnification)
		Rostral Infundibulum: Central linear defect with a thin rostrobuccal extension - Diameter: 1.12 mm Caudal Infundibulum: Multiple, focal, small hypointense areas in region of central vascular channel; within normal limits	Rostral Infundibulum: Discoloured cementum in the region of the central vascular channel and extending into rostro-buccal infolding Caudal Infundibulum: Very small area of slightly discoloured cementum in the region of the central vascular channel	• Prominent vascular channel branches filled with cellular debris at periphery of infundibular cementum • Wider, empty vascular channel branches closer to the central region • Halo of enamel remnants following decalcification (E)

3.4.1 Density of infundibular contents in Hounsfield Units

Cementum of normal gross appearance assessed in selected microCT images was found to have to have a mean density of 3222 Hounsfield units (HU), ranging from 2029 to 3725 HU (Range = 1696 HU). The mean density of defective regions of cementum was 401 HU, with a much wider range from -692 to 1343 HU (Range = 2035 HU).

Histological examinations of the above specimens revealed extensive differences in the structure of the cementum and in the debris or lesions found within different infundibulae, some which had a similar appearance on both on microCT and gross examination. MicroCT density measurement (in HU) of infundibular cementum at various depths and the histological findings at these sites are summarized in Table 3.4.3.

Table 3.4.3: MicroCT density (HU) of cementum with a normal appearance on microCT images (HU of Cementum) and areas which appeared to be hypointense, representing lesions found within infundibular cementum (HU of Lesions) of 8 maxillary cheek teeth and histological findings of the corresponding cementum.

Tooth	Section Depth	Infundibulum	HU of Cementum (microCT)	HU of Lesions (microCT)	Histological Description of cementum
A	30 mm	Rostral	3438	99	• Many branches from the central vascular channel • Fragments of cementum mixed with plant material in vascular channels
		Caudal	3578		• Sharply defined border of central vascular channel • Little material within the vascular channel
	40 mm	Rostral	2815	22	• Hypoplastic cementum • Plant material within cemental defects in buccal infoldings
		Caudal	3364		• Sharply defined border of vascular channel • Vascular channel empty
	55 mm	Rostral	2029		• No cementum present • Small amount cemental debris
		Caudal	2813	537	• Extensive lateral vascular channel branches within cementum • No debris apparent in channels

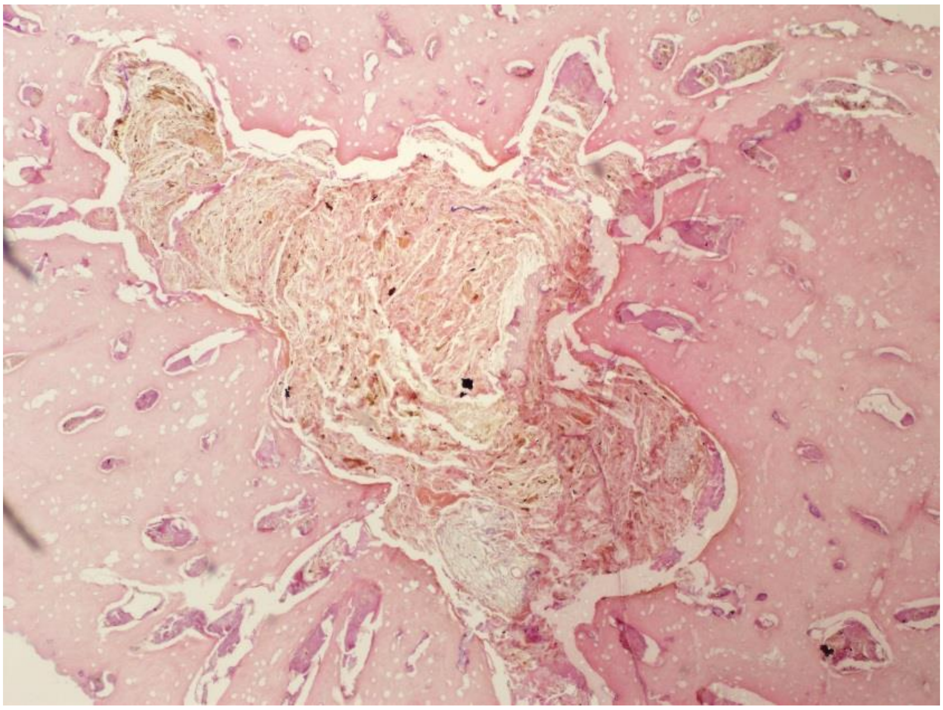
B	20 mm	Rostral	3655	671	• Long lateral vascular channel branches • Pale cellular debris in central vascular channel
		Caudal	3696		• Long lateral vascular channel branches • Pale cellular debris in central vascular channel
	50 mm	Rostral	3244	349	• Many lateral branches off central vascular channel • Fragments of cementum mixed with plant material in branches of vascular channel
		Caudal	3529		• Very narrow vascular channel
C	5 mm	Rostral	3511	1161	• Artefactual cemental fracture • Plant debris with cellulose structure in central vascular channel
		Caudal	3725	1343	• Artefactual cemental fracture
	15 mm	Rostral	3145	475	• Many branched, empty vascular channels
		Caudal	3692	1132	• Many food filled vascular channels in cementum
D	30 mm	Rostral	No cementum visible on CT	-396	• Moth-eaten cementum containing debris • Basophilic pockets of mineralisation
		Caudal		-560	• Strands of collagen in mid-infundibulum
	40 mm	Rostral		-517	• Absence of cementum • Fibrillation or pellicle of cementum present
		Caudal		-692	• Cementum appears to be degenerating
E	15 mm	Rostral	Abnormal appearance	1265	• Basophilic stippling within cementum • Contains brown plant material
		Caudal	2158	717	• Well-defined central vascular channel • Food material in vascular channel
H	10 mm	Rostral	2931	805	• Many vascular channels in cementum • Dark plant debris in central vascular channel but not in branches
		Caudal	3448		• Many empty vascular channels in cementum

To examine possible reasons for the wide range of HU measurements in defective cemental areas within the infundibulae, abnormal infundibulae were separated by histological assessment into infundibulae with cementum infiltrated by plant material and infundibulae with some degree of cemental hypoplasia without plant material. The mean density of the cemental areas containing plant material was 694 HU (Range: 22-1265 HU), while the mean density of areas with cemental hypoplasia was -70 HU (Range: -692 to 671 HU).

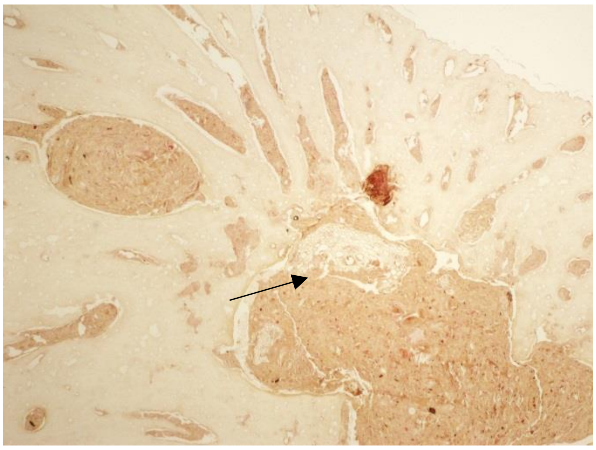
Examination of all histological samples (H&E stained) for evidence of either endothelial lining cells or haemosiderin (evidence of recent live vasculature) identified three sections (from teeth A, C, E) as containing brown or red coloured material which could possibly be haemosiderin or material of plant origin. However, Prussian Blue staining revealed no haemosiderin to be present in the sections examined (Figure 3.4.3). None of the histological samples examined revealed evidence of recent viable vascular tissue in these infundibulae.

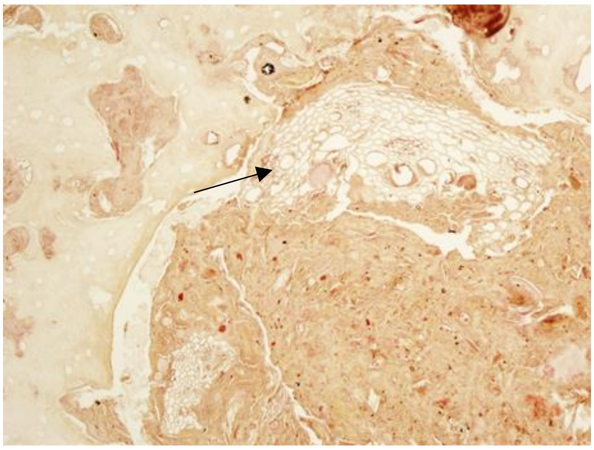
Figure 3.4.3:

1) H&E stained section of central vascular channel of rostral infundibulum of tooth A (4x magnification) that had visible occlusal caries showing an irregularly shaped channel filled with plant material.



2) Prussian Blue stained sections of the central vascular channel of the rostral infundibulum of tooth A (4x and 10x respectively); note the cellulose (plant) structure at the arrow.





4. Discussion

Maxillary cheek tooth infundibular caries is one of the most common dental disorders reported in equidae and can lead to severe complications such as midline fractures with or without apical infection and secondary sinusitis. Infundibular caries has been found in a high percentage of maxillary cheek teeth in many previous studies, with a reported prevalence of 65% in a computed tomographical study (Windley et al 2009b) and a 97% prevalence on gross examination (Honma et al 1962).

In this study, a great majority (68%) of infundibulae were found to have occlusal surface lesions, but an even more significant majority (91%) were found to be affected by subocclusal cemental lesions. Even infundibulae which appeared to have no evidence of cemental defect on the occlusal surface were affected by occult subocclusal cemental lesions. There also appears to be no relationship between the appearance of the occlusal surface of the infundibulum and any subocclusal cemental lesions. Many teeth which appeared to have no visible occlusal lesions also contained impacted feed material at the most apical aspect of the infundibulum, indicating that there was some communication with the occlusal surface in teeth which would also be categorized as completely normal on occlusal surface examination. As so many occlusal and subocclusal lesions were present within infundibulae of teeth with no other significant pathology found, a redefinition of normal infundibular cementum and classification of subocclusal infundibular defects may be required along with a better understanding of their relationship to clinical sequelae such as tooth fracture or sinusitis.

Although the relationship was not able to be followed in vivo in this study, we would propose that the most severe infundibular caries actually form subocclusally, prior to exposure of the diseased cementum on the occlusal surface. If areas of cemental hypoplasia are exposed to the oral environment through mild (Grade 1) surface caries or patent vascular channels, and then become impacted with feed material, the process of cemental degradation and caries formation may begin long before the tooth is worn to that point. These severe caries may form long before they can be identified on standard dental evaluation, and may require more advanced imaging techniques, such as CT scan, to be discovered prior to causing clinical sequelae. The use of density measurements on microCT scan (Hounsfield Units) in comparison with histology has allowed us to better understand the appearance of food material, hypoplastic cementum, and normal cementum on CT scan. These results may be extrapolated in the future and be applied to standard CT scans of infundibulae to understand what tissues and substances are below the occlusal surface prior to tooth eruption.

4.1 Sample Selection and Assessment

The use of cadaver teeth (that can only be examined on single occasion) as opposed to repeated monitoring of clinical cases prevented assessment of the progression of this disease which is known to occur (Marsh and Martin 2004). Teeth were placed immediately in formalin, however it took several weeks for the teeth to completely fix. As the infundibulae were the most central portion of the teeth and would possibly take the longest time to fix in formalin, it is possible that some further tissue degradation occurred during this process in the infundibular defects which had some connection with the occlusal surface. Formalin also ensured that the feed material packed within the infundibulae was sterile when the teeth were sectioned when some of the contents (including possibly carious dental material, food material, and bacteria) within the infundibulae were invariably aerosolized, which was beneficial from a health and safety perspective.

A range of teeth with and without visible occlusal lesions were selected from the 30 cadaver skulls, including from 1 to 12 maxillary cheek teeth per cadaver skull. Twenty-four cadaver skulls contributed between 2 and 4 teeth each to the study population. The very limited availability of horses between 1 and 4 years old with erupting cheek teeth necessitated 12 maxillary cheek teeth from a 3.5 year old horse being used in order to examine the developing and recently developed infundibulae. Interestingly, a description of cemental deposition along the central vascular channel of the infundibulum immediately prior to and after eruption of the tooth has only been recently published (Suske et al 2016a).

One limitation in obtaining teeth without visible occlusal abnormalites was that so few skulls were totally free of infundibular lesions, that 16/18 control teeth were taken from cadavers in which other teeth were affected by visible occlusal infundibular caries. Additionally, 14/18 teeth (18/36 infundibulae) without occlusal caries that were selected as ‘unaffected’ samples were found on CT scan to have subocclusal lesions, which in this case were developmentally incompletely filled with cementum either as central linear cemental defects or apical cemental hypoplasia. The ‘unaffected’ samples in this study therefore represent those teeth with a normal infundibular occlusal surface that clinically would be deemed to be unaffected by caries. Such teeth, with a normal occlusal surface and deep infundibular cemental defects, would *in vivo* eventually have exposure of their deep cemental lesions as the teeth erupted and wore. Teeth that previously were deemed unaffected would then have visible occlusal lesions once that had worn to that level. Once again, this demonstrates the difficulty of using cadaver samples to represent a progressive disease. It also indicates that further studies are needed to follow the progression of infundibular caries in clinical cases. The analysis of peripheral caries, thought to be another progressive and common dental disorder in equids, is outwith the scope of this study, and will also require much further research in clinical cases to determine how and why it may progress. Peripheral caries were therefore not commented on in regards to the samples chosen for this research.

The teeth selected included a range of cheek teeth from each Triadan cheek tooth position (06-11). The 09 teeth were overrepresented understandably, as this tooth has been shown to be most likely to be affected by caries and midline sagittal fracture in various studies (Dacre et al 2007; Fitzgibbon et al 2010; Veraa et al 2009; Dixon et al 2014). The least number of teeth were taken from the 06 and 11 Triadan positions (n = 4 and 5, respectively). The 11s were least likely to be affected by infundibular caries in other studies (Fitzgibbon et al 2010), however they have been found to be the most affected by apical cemental hypoplasia (Dacre et al 2008a).

To reduce the potential inaccuracy of ageing by dental examinations, horse age was recorded in five-year age ranges. In this study, the *age of the horse* rather than the *dental age* of each tooth was recorded. Eruption times of the permanent cheek teeth have been noted previously. Therefore, the horse's recorded age varies from the dental age of each individual cheek tooth. Four of the samples were obtained just prior to their eruption, and would have appeared in the analysis as a negative age if dental age were used as opposed to age of the horse.

Teeth were selected for the study to ensure a similar number was obtained from each age group, but this necessary step precludes any age-related statistical analysis. The least number of samples were obtained from both extremes in age range, with 14 teeth obtained from horses under 5 years in age and 17 teeth obtained from horses over 20 years in age. Often in aged horses, the infundibulae wear out completely, leading to a condition known as 'senile excavation' (Dixon et al 2013). In such teeth, there is no infundibulum left to assess and such senile teeth were excluded from the study, limiting the number of aged horses that were available. The group with the least representation was with horses under 5 years of age. In addition to fewer young cadavers being available for sampling, these younger teeth would have been in wear for a much shorter period of time and be less likely to have occlusal infundibular lesions. Some subjectivity had to be applied when grading the caries in younger teeth because incomplete cemental filling is often present in the central aspect of the wide infundibulum that is present when the tooth erupts, along with enamel cusps that protrude prominently (Dacre 2005). This normal, temporary feature could be confused with deep cemental caries.

Due to the selection of teeth by observers and from specific age groups, there is invariably a sampling bias involved in this survey. As the occlusal surface of the tooth was seen prior to the selection of the samples, the number both with and without visible occlusal surface lesions was chosen. This bias invariably affects the validity of the data when applied to the population as a whole, and limits the use of any prevalence data regarding visible occlusal surface lesions as the selection may have led to an over- or an under-estimation of the prevalence of infundibular caries or a particular caries grade. The presence and type of any subocclusal lesions were unknown prior to selection of the teeth, and their relationship with any visible occlusal lesions may involve less bias. However, any statistical significance attached to this data must be viewed and interpreted prudently.

4.2 Occlusal Surface: Infundibular Caries

Infundibulae were determined to have caries on gross examination of their occlusal surface if the area of degradation or discoloration surrounding the infundibular central vascular channel was greater than 1 mm in diameter (C Staszyk, personal communications 2015; Suske et al 2016b). This is an arbitrary measure of determining if an infundibulum is affected by caries as opposed to simply having a wide central vascular channel. There is some discrepancy in the terminology of this disorder as Suske et al (2016b) terms occlusal ‘cemental hypoplasia’ to include central vascular channels >1 mm in diameter, while the term ‘infundibular erosion’ involved acquired change in the structure of the infundibulum (Suske et al 2016b), indicating that the acquired changes may not involve bacterial degradation. The term ‘infundibular erosion’ as used by these authors seems to encompass complete Grade 1 infundibular caries, and also the Grade 2 and Grade 3 caries as described by Dacre (2005) and used in the present study. Regarding infundibular erosion, which does not involve bacterial degradation, there is no evidence that this is the disease process occurring within the infundibulae as opposed to caries formation involving bacteria, The term ‘cemental hypoplasia’ of the occlusal surface as used by Suske et al (2016b) is similar to Grade 1 caries in the more commonly used terminology (Dacre 2005). For many years, ‘cemental hypoplasia’ has been used to describe a lack of cementum subocclusally only, on both CT scan and gross examination (Dacre et al 2008a; Veraa et al 2009; Fitzgibbon et al 2010). However, cemental hypoplasia may affect the occlusal surface of the tooth as well as the subocclusal and apical regions.

The Modified Honma Grading system (Dacre 2005) does not clearly separate those Grade 1 caries (caries involving cementum only) which affect only a localised region surrounding the central vascular channel from caries that affects all of the infundibular cemental filling, making ‘Grade 1 caries’ describe a wide range of severity of cemental caries. Instead of making the differentiation of and terminology regarding hypoplasia, caries, and erosion even more complex, a system of sub-classification of Grade 1 caries could be used in the future to describe the extent of changes to the infundibular cementum in more detail. We propose that Grade 1a infundibular caries describe those with <50% of the occlusal infundibular cementum affected, with changes confined to the area around central vascular channel, while Grade 1b would indicate >50% of the occlusal infundibular cementum was affected and beginning to reach the margins of the infundibulum. However, any lesion in the infundibular cementum, possibly even less than 1 mm in diameter creates the propensity for these vascular channels to fill with food material which can then act as a bacterial substrate to allow caries to develop and degrade the surrounding cementum. While wide central vascular channels or mild Grade 1 infundibular caries are not an immediate cause for concern, and in fact are defined as a variation in normal anatomy by some (Suske et al 2016b), they can allow food ingress, and then later support a pathological process to proceed subocclusally.

There was a statistically significant difference in the prevalence and in the severity of occlusal surface caries between the age groups of the sample teeth, with a trend towards more frequent and severe caries being more commonly found in older horses. It is interesting that the youngest age group (<5 years old) was completely unaffected by Grade 2 and 3 caries, but there were also some teeth from the older age groups completely unaffected. This indicates some teeth may be completely resistant to infundibular caries formation. The most likely scenario being that if maxillary cheek teeth are completely devoid of cemental defects, then food material will not become impacted subocclusally, and infundibular caries cannot develop. Although we did not observe the dentition of these older horses without infundibular caries *in vivo*, it is likely that these more resilient teeth were unaffected by occlusal caries and/or central linear defects when younger, as the deeper cementum remained lesion-free. If these infundibulae had occlusal defects that allowed entrapment of food material in the infundibulae, it is likely that the infundibulae would have developed significant infundibular caries by the time of our examination up to 20 years later. This may indicate that dental caries, and infundibular caries in particular, is not a progressive disease as previously thought. Though the degradation of the dental tissue advances and progresses over time, it is possible that a predisposition for more severe caries, in the form of an apical cemental lesion, must exist for infundibulae to be affected by Grade 3 or even Grade 2 caries over time, potentially along with communication of this apical lesion with the occlusal surface. Infundibular caries may then be considered a disease with multiple components as opposed to a purely progressive disease.

There was a significant difference in the overall prevalence of occlusal caries between Triadan positions with the 07 and then the 09 infundibulae most commonly affected. Additionally, the caries grade differed significantly between Triadan positions, and the high number of Triadan 09 teeth included in the study likely influencing this value. The 11 position was the only one with a minority (30%) of infundibulae affected. Veraa et al (2009) indicated that the 09 and 10 cheek teeth were most affected by caries, and Fitzgibbon et al (2010) also found the 09 teeth to be most affected while the Triadan 11 teeth were least affected by caries. In the current study, the 09 position was affected most by Grade 2 and 3 caries in comparison to the other positions, while the 06, 07, and 11 teeth were rarely affected by more severe caries. These other studies did not report an increased prevalence in caries of the Triadan 07 cheek teeth as found in this study, but few Triadan 07 teeth were included in this study (n=8) as opposed to many more Triadan 09 Teeth (n=47). The significance of the high prevalence of caries in the 07 teeth (7/8; 88%), along the prevalence of caries in the 06 and 11 cheek teeth (63% and 30% respectively) must be cautiously considered in light of the low numbers of 06, 07, and 11 Triadan teeth included in the analysis.

Previous studies (Veraa et al 2009; Windley et al 2009b; Suske et al 2016a) have shown that the rostral infundibulae of maxillary cheek teeth are more likely to be affected by developmental cemental defects and caries than the caudal infundibulae. Recently, Suske et al (2016a) have described lateral branches of the infundibular blood supply that appear to persist for a longer period in the caudal as compared to the rostral infundibulum, and consequently, the rostral infundibulum tends to have a lower cemental content predisposing to disease. The current study also found the rostral infundibulae more commonly affected (72%) than the caudal (62%) infundibulae, but this difference was not statistically significant. Both infundibulae were affected in 54% of the teeth sampled and in the 28 teeth in which only one infundibulum was affected, more rostral infundibulae (18/28) than caudal infundibulae (10/28) had occlusal caries.

There was, however, a significant difference in the severity of caries between the rostral and caudal infundibulae, with the rostral infundibulum being affected by more severe lesions. Fifty-two percent of the teeth were affected by the same grade of caries, but the rostral infundibulae were more severely affected in 34% of cases. This could be related to the earlier loss of the lateral blood supply to the rostral as compared to the caudal infundibulum (Suske et al 2016a).

4.3 Computed Tomography

Veraa et al (2009) described hypoattenuation, or reduced radiodensity, of the infundibulae to be present in some maxillary cheek teeth of all horses imaged on CT scan (52% overall prevalence), but no further classification of infundibular defects were presented. Windley et al (2009b) categorised defects in infundibular cementum recognized on CT scans into cemental hypoplasia (both at the amelocemental junction and centrally around the vascular channel) along with infundibular caries. In the current study, many differing infundibular lesions were imaged on CT scans that could be considered as being caused by either cemental hypoplasia or caries. However, based on pilot studies and the findings of Fitzgibbon et al (2010) the subocclusal infundibular lesions imaged on CT scan were classified as either caries, central linear cemental defects, or apical cemental hypoplasia.

Caries was identified on CT scan by the intensity of the material found within the infundibulum, with cemental caries appearing as hypointense in comparison to normal cementum. This is likely due to the degraded or developmentally absent cementum along with food material found in carious regions. In turn, areas of cemental caries were hyperintense in comparison to infundibular areas with defective (partial or total) cemental filling (cemental hypoplasia). Central linear cemental defects were identified as having a hypointense area surrounding the linear central vascular channel that was >1 mm diameter, as described earlier, as opposed to a non-significant central vascular channel remnant (<1 mm diameter).

In this study, 49% of infundibulae that were affected by occlusal surface caries were also subocclusally affected by central linear defects. However, an even greater proportion (64%) of infundibulae unaffected by occlusal infundibular caries were affected by subocclusal central linear defects alone. The Triadan 09 position, which is recognised as the Triadan position to most likely to be affected by infundibular caries, was the second least common Triadan position (47% prevalence) to be affected by central linear defects. Additionally, it was found that the teeth of younger horses were more likely to be affected by central linear defects than those of older horses. A possible reason for this is that central linear defects play a role in the development of caries over time as food become impacted into and cementum becomes carious around the central vascular channel and thus in older horses would appear as subocclusal caries.

Cemental hypoplasia was identified when a majority of the width of the infundibulum was incompletely filled with cementum in a particular section. Cemental hypoplasia, in theory, could occur at any location along the infundibulum, but was mainly found at the apical aspect of the infundibulum and typically extended occlusally only to the junction of the apical and middle third of the infundibulum. This defect, termed cemental hypoplasia, should not be confused with a more complete hypoplasia of the infundibular apex where the base of the enamel infundibular ‘cup’ did not develop, as described in a small number of cases by Dacre et al (2008a) and Pearce (2015). No such generalised infundibular apical defects were found in this study.

Apical cemental hypoplasia of infundibulae occurs more commonly in infundibulae concurrently affected by other lesions more occlusally (central linear defects or mild caries), and it can even be found deep to completely normal occlusal surface cementum. Another recent study proposes that this apical cemental hypoplasia be termed ‘cemental aplasia’ and that ‘cemental hypoplasia’ be used to describe both occlusal surface cemental defects along with central linear cemental defects (Suske et al 2016b). However, as cemental aplasia means total absence of cementum, this is not an accurate term because there is still some cemental filling in these infundibulae occlusal to the “aplastic” region. Additionally, on histological examination, areas that appear to be completely devoid of cementum actually contain either moth-eaten or fibrillated cementum that cannot be seen grossly or on CT scan. Multiple previous studies have used the term cemental hypoplasia to describe an area of the infundibulum with cemental filling defects, and this terminology was used in the present study.

Most (81%) infundibulae with occlusal surface caries were unaffected by apical cemental hypoplasia. Infundibulae affected by apical cemental hypoplasia were unaffected on the occlusal surface (47%) or were affected by Grade 1 occlusal infundibular caries (49%). Infundibulae with more severe (Grade 2 or 3) occlusal caries were rarely affected by apical cemental hypoplasia (4% and 0% respectively). This is not surprising because in infundibulae with more severe occlusal caries, the caries often extends to the apex of the infundibulum, and if cemental hypoplasia was initially present, it would no longer be recognisable once the area was affected by caries. This finding further indicates that the appearance of the occlusal surface is not predictive of subocclusal infundibular disorders and defects.

There was no significant difference in the prevalence of cemental hypoplasia between age groups. Even in the oldest of horses and teeth examined, regions of hypoplasia restricted to the area of the apex of the infundibulum may never become exposed. Additionally, variations in eruption time between horses and in the 5-year range included in the age groups may have affected interpretation of this analysis statistically. However, there was a steady age-related decline in the proportion of those teeth affected by cemental hypoplasia, with the highest prevalence in the <5 year age group (32%) and the lowest in the >20 year group (15%). Despite not being statistically significant, this is once again an inverse relationship to the prevalence and severity of infundibular caries. It is likely that areas of apical cemental hypoplasia are exposed to the occlusal surface as the tooth wears and then become impacted with food and develop caries and are later identified as having occlusal infundibular caries on clinical examination, as proposed by Dacre (2005). Infundibulae affected by the most severe caries in older horses may be those that have had a central linear defect combined with a large area of apical cemental hypoplasia. Food material and bacteria may enter deep into the infundibulum through a patent vascular channel or wider central linear defect early during the life of the tooth and begin to degrade any remaining cementum at the apical aspect of the infundibulum, as noted above. With continuous inoculation of food material and bacteria from the oral cavity through even the smallest of occlusal cemental lesions, linear defects, and cemental hypoplasia may then progress to caries of the surrounding enamel and even dentine, again meaning that caries is likely a disease with multiple components.

The location of defects were grouped as occlusal, meaning the lesion involved the occlusal surface of the infundibulum, or as subocclusal. The latter were divided into apical, meaning the lesion was found in the apical-most third of the infundibulum and reached the apical enamel margin of the infundibulum, and mid-infundibulum, essentially meaning it was found in the region between the apex and the occlusal surface of the infundibulum. In theory, any disorder may occur in any region of the infundibulum, however the cemental hypoplasia found in our study population was more likely to be confined to the apical region, and infundibular caries was more likely to occur in the occlusal region. Central linear defects may occur anywhere along the length of the tooth, and caries may traverse deeper than the occlusal surface if severe. Caries cannot occur in the apical or mid-portion of the infundibulum without also occurring on the occlusal surface. The absence of visible occlusal infundibular caries of course did not preclude the tooth from having developmental cemental lesions including linear cemental defects or deeper cemental hypoplasia. Each individual infundibular lesion and its location was recorded to allow cross-analysis and comparison. However, interpretation of comparison must take the most logical combinations of lesions and location into account.

A vast majority of infundibulae (80%) and teeth (87%) in this study were affected at their apical aspect by some type of lesion. There were significant differences in apical cemental lesion prevalence between the Triadan position of affected teeth and whether the affected infundibulum was rostral or caudal within the same tooth, but a great majority of apices were affected by cemental defect regardless of the Triadan position of the tooth or infundibular position, with the exception of the Triadan 11 teeth. Overall, 84% of teeth with caries had an apical cemental lesion, including caries reaching the apical extent of the infundibulum, and teeth with more severe caries were more likely to have an apical lesion, including 93% of those with Grade 3 caries as opposed to 80% of those with Grade 1 caries.

The exception to many of these findings regarding infundibular caries and cemental defects in general is the Triadan 11 tooth. Although only 5 Triadan 11 teeth were included in the study, it was the only Triadan position in which the majority of teeth were unaffected by any infundibular lesion. The 11 tooth is one of the youngest permanent teeth in the body, and it appears from these low numbers of samples that the Triadan 11 position is possibly predictive against infundibular abnormalities.

Additionally, as expected, the ID:CL ratio decreased in older horses as the crown wore and the infundibulum became proportionally shorter in relation to the entire crown length. The median ID:CL ratio varied from 88% in the horses <5 years in age, to 39% in horses >20 years old. Similar studies on gross sections by Fitzgibbon et al (2010) showed the ID:CL ratio to vary as much as from 99% in a 4-year-old to 7% in a 16-year-old horse. In this study, the ID:CL ratio was lowest in the Triadan 09 position (57%), which would be expected as the 09 is the oldest tooth in the body and has been in wear for a longer period than all other cheek teeth. Additionally, the ID:CL ratio decreased as the caries grade increased, from 74% in unaffected teeth to 44% in infundibulae affected by Grade 3 caries, which corresponds to many 09 infundibulae being affected by Grade 3 caries (9/94; 9.5%). The relationship of the ID:CL ratio to age of the horse, and the relationship of the ID:CL ratio to caries grade, are supportive of the relationship between age and caries grade, though the ID:CL ratio in itself likely is not the most useful tool clinically in predicting caries formation or infundibular disease.

4.4 MicroCT and Histology

Histological examination of equine teeth is difficult process, with the necessary decalcification procedure always causing loss of enamel and sometimes more extensive disintegration of the specimen. Therefore, a more novel biomedical imaging technique, micro-computed tomography (microCT), was used to assess the structure of the equine cheek tooth in great detail without disruption of the physical tooth structure.

In the past 15 years, microCT has been used in human experimental dentistry to examine the apical canal volume and geometry during and following root canal treatment procedures (Paqué et al 2009; Rhodes et al 1999). MicroCT functions in a similar way to the more commonly used volume imaging of a clinical helical full-size CT scanner but on a smaller scale. The use of high intensity x-ray sources, however, allows the photon energy to be adjusted specifically to correlate to the diameter of the specimen, which typically determines the attenuation of the signal transmitted. Whereas the standard CT scanner scans volumes of 1 mm^3, a microCT scanner can image sections as small as 1 -

5 micrometres[3], and is essentially able to achieve subcellular dimensions in certain circumstances (Ritman 2004), though this was not the case in this study. Images obtained from microCT studies can then be used to direct the sectioning and histology of teeth to areas of interest, instead of sectioning blindly and possibly missing an area of interest if for example, it was only a few mm thick. Micro-computed tomography, however, is limited by the size of the specimens as the small field of view of most scanners that can only accommodate specimens 70 mm or less in length, necessitating the extraction of teeth prior to imaging, which of course prevents its use in clinical cases.

MicroCT utilizes computed tomographic imaging with increased resolution that in turn allowed extremely detailed three-dimensional microscopic images of equine cheek teeth to be made. This technique allowed very detailed analysis of the complete cheek tooth including infundibular cementum, enamel, and adjacent dentine. Images obtained from micro-computed tomography of diseased cheek teeth were used to select areas of interest for gross and histological examinations whose findings were then compared to the Micro CT findings at these chosen sites. The 8 teeth that underwent microCT imaging and were later sectioned using microCT images for guidance. This allowed the comparison of appearances of normal, carious, and hypoplastic cementum on CT scan, gross visual examination, and histological section. Little consistency was observed between conventional computed tomographic and MicroCT images. For example, the appearance of a standard CT scan would just suggest that an infundibulum was devoid of cementum, but upon examination grossly and on microCT the infundibulum would appear to be filled with plant material. On microscope evaluation of the slides however, the infundibulum was found to frequently to contain porous cementum in addition to plant material. Overall, the gross and imaging appearance of the infundibulum on microCT imaging was a relatively inaccurate predictor of the histological appearance of hypointense infundibular contents, although the technique was excellent for imaging the dental tissues alone.

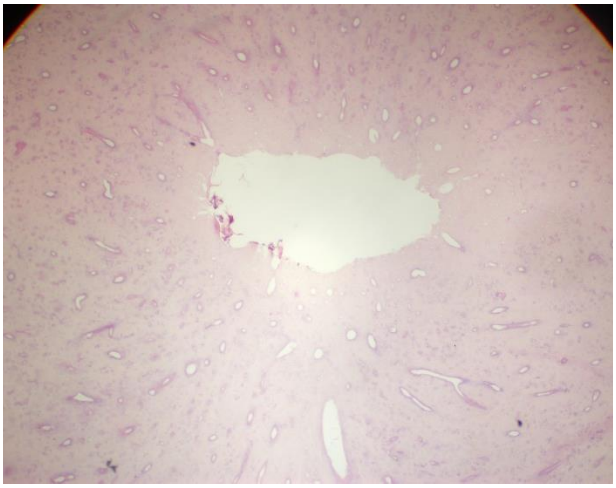
Similar infundibular abnormalities were identified with both microCT and standard CT scan, however these lesions were much more subtle on microCT than our three basic categories (central linear defects, cemental hypoplasia, or caries). MicroCT images allowed more detailed subjective evaluation of the infundibular structure of each tooth compared to the standard CT images, and also allowed measurement of the attenuation or density of the tissues imaged in each plane. In particular, areas that were thought to be completely devoid of cementum (complete apical cemental hypoplasia) were found to contain at least some evidence of cemental filling and HU obtained for these areas indicated that the infundibulae were not completely devoid of cementum.

Confirming the suspicion obtained on microCT imaging, all infundibulae were found to contain either discoloured cementum and/or necrotic feed material when sectioned that was typically concentrated around the central vascular channel. In addition, this finding often held true even when an area of infundibulum appeared to be completely empty on microCT. Infundibular cemental discolouration was much more prominent in some teeth than others, and appeared to be related to the type of defect imaged in each infundibulum. In general, the appearance of infundibular contents on microCT images was a relatively poor predictor for their gross or histological appearance, especially within and surrounding the vascular channel (see Figure 4.2).

The material found within the infundibulum did not seem to be dependent on the level within the infundibulum in which it was located. Discoloured material was found near the occlusal surface, in the mid-infundibulum, and at its apical aspect. Discoloured cementum and food was often found deep within infundibulae that appeared to be normal on the occlusal surface (caries Grade 0), which means that a patent vascular channel must have still been present and open to the occlusal surface of the tooth despite its gross appearance.

In only one tooth did both infundibulae appear to be truly devoid of any cemental or food filling grossly and histologically. The remaining 14 infundibulae histologically examined were filled with some cementum that varied in quality and quantity between infundibulae. On histology, infundibulae that appeared to have grossly normal cementum on both microCT and gross sectioning contained an obvious central vascular channel in the cementum along with multiple horizontal branches extending peripherally through the cementum, including into the buccal infoldings (Figure 4.1).

Figure 4.1: Histological sections of infundibular cementum containing a central vascular channel and multiple lateral branches running horizontally through the cementum (2x and 4x respectively).

These branches were either completely empty or contained pale cellular debris. Infundibulae which appear to have a central vascular associated defect or a large area of apical cemental hypoplasia on microCT and that contained discoloured cementum and/or feed material on gross section often still had the expected infundibular architecture with a central vascular channel and multiple lateral branches. However, the central channel was often wide and filled with food material, as were the lateral branches, thus containing much less cementum than normal. The cementum in such lesions was occasionally fragmented and mixed with food material. Infundibulae which appear to be completely empty on microCT and gross section sometimes contained moth-eaten cementum or only a few strands of soft tissue suspected to be collagen that had peeled off the amelocemental junction.

Kilic et al (1997b) found similar infundibular cemental architecture histologically, with up to one-third of the cementum section being composed of these tortuous vascular channels, particularly in areas that appeared to have cemental hypoplasia. Patent central vascular channels were also found at the occlusal surface in 65% of teeth. Kilic proposed that if these vascular channels contacted deeper areas of cemental hypoplasia or other cemental defects, this would allow entrapment of feed material and degradation of infundibular cementum below the occlusal surface, as suggested earlier. The histological findings in the current study support this theory.

In conjunction with CT and microCT findings, the current gross and histopathological findings support that often the most severe infundibular caries are due to the subocclusal degradation of infundibular cementum, enamel, and eventually dentine. In this study, the most severe caries was recorded in horses <15 years old instead of as expected, in the older horses. This finding may indicate that it is not simply a progressive caries-induced degradation of cementum that eventually leads to the most severe infundibular caries, but that a developmental cemental defect is present in infundibulae that develop severe caries at a younger age. In teeth with marked cemental hypoplasia, especially if present in the apical or mid-infundibular area, or even those with extensive linear defects, feed impaction through subclinical cemental defects in the occlusal surface cementum may allow severe subocclusal caries to form well ahead of their appearance on the occlusal surface. Additionally, it has been proposed that necrosis of the former vascular tissue and channel endothelial lining would provide additional substrate for bacterial growth (Kilic et al 1997b).

The current findings confirm that food material, which could serve as a bacterial growth substrate, may be present several centimetres below the occlusal surface even in teeth that appear to be unaffected on clinical (occlusal) and CT examinations, but obviously are affected by a subclinical central linear defect. Therefore, the most severe caries may form prior to exposure of the affected hypoplastic cementum, and detection may not be possible by regular dental examination. Imaging which allows investigation of the subocclusal aspects of the infundibulum may be needed to determine the most at-risk teeth, but this cannot be determined with certainty until *in vivo* studies over a course of many years are performed on this disorder.

In this study, examinations to detect viable blood vessels in the infundibular cementum following eruption were unsuccessful, with no evidence of the presence of vascular endothelial lining cells at the apical aspect of any infundibulae. This is in contrast to previous reports by Dixon and DuToit (2010) in which evidence of viable apical vasculature was found even after the teeth had erupted. As noted, more recent research performed on maxillary cheek teeth immediately prior to eruption found no evidence of apical vasculature (Suske et al 2016a). Apical enamel formation (amelogenesis) occurs prior to cementogenesis, and there is currently no evidence that equine dental vasculature to the infundibulum exist prior to amelogenesis. It is unlikely that vessels could penetrate the fully formed enamel at the apical-most aspect of the equine infundibulum during cementogenesis either, which then limits the cemental blood supply to the central vascular channel and more occlusal structures (Suske et al 2016a). Despite cementogenesis supposedly being confined to the occlusal surface region, lateral branching from the central vascular channel was observed at all levels histologically in this study, indicating the presence of an extensive blood supply from the main occlusal and smaller lateral vasculature proceeding in an apical direction prior to the eruption of the tooth.

To further search for evidence of recent vascularization of the apical infundibulum, any sections that contained brown or red-coloured material on H&E staining were also submitted for staining with Prussian Blue pigment. This pigment identifies any iron released from the degradation of haemoglobin, such in the form of haemosiderin (Sundberg and Broman 1955), however none was found in the samples examined. This contrasts with another recent study in which 20% of recently erupted maxillary cheek teeth infundibulae were found to contain porphyrin, another product of haemoglobin degradation, at varying times following eruption (Suske et al 2016b). In the current study, 6 of the 8 teeth examined by microCT and histology had been in wear for many years, but one tooth (Tooth C) had been in wear for less than one year and would have been the most likely to still contain viable vasculature. Additionally, evidence of blood products would be present within the central vascular channel or its lateral branches if a recent blood supply had been present in the area of the infundibulum examined on histology. A proposed theory is that the blood supply to the apical portion of the infundibulum is lost if cementogenesis occurs too quickly at the occlusal surface of the infundibulum effectively pinching off the central vessel prematurely (Suske et al 2016a).

MicroCT images were also used to measure the density or attenuation of normal and defective cementum within each infundibulum. Each pixel in a CT scan image is assigned a numerical value that corresponds to the attenuation of the x-rays caused by passing through the tissue being imaged. The 'Hounsfield Unit' is assigned to compare the attenuation of the imaged tissue to water (HU = 0), and each subsequent unit represents 0.1% of the attenuation of water (Brooks and Chiro 1976). Tissue attenuation is a measure of how easily a tissue may be penetrated by the x-ray beam, with higher attenuation meaning the beam is unable to pass through as easily as in tissues with lower attenuation. Air has a density measure of -1000 HU, while bone can range from 700 to 3000 HU. Variations in the shades of grey on the CT image are often used to distinguish between fluid or soft tissue, and bone or enamel. However, without HU measurements, the human eye would not be able to distinguish between approximately 4000 different shades of grey, and particularly would not be able to distinguish between the density of the filling within the different infundibulae. Tissues measured to be (+) or over 0 HU are more dense or more attenuating than water, such as bone or soft tissue, while those that are (-) or under 0 HU are less dense than water, such as fat or air (Keil and Heverhagen 2008). A study on donkey teeth found the mean HU of infundibular cementum to be 1852, while enamel was 3022, primary dentine was 2196, and pulp was 387 (du Toit 2009).

In this study, the HU measurements found for infundibular cementum on the microCT images were much higher, on average, than the standard CT reported HU values for cementum. On microCT, tissues similar to the attenuation of water, such as soft tissue and fluid, have similar HU to those on helical CT. However, materials that have a higher mineral content and density than water, such as bone or enamel, react very differently when absorbing radiation in a different pattern and possibly on a different spectrum than the helical CT scan (Liu et al 2013). Therefore, the HU for bone on a microCT can reach up to 7000 HU, and it is not surprising that the mean HU of cementum on microCT in this study (3222 HU) was much higher than recorded in equidae cementum on standard CT. Interestingly, the mean HU of the defective regions of cementum was 401 HU, which is similar to the HU value for soft tissue on a standard CT scan.

The range in HU of infundibular cementum of normal appearance on microCT was broad, from 2029 to 3725 HU. A range this broad (1696 HU) in a bony structure would indicate that different types of bone are present, such as cancellous and cortical bone (typical range of 2000 HU between these two types of bone on microCT). The wide HU range for equine infundibular cementum indicates that the cemental structure differs markedly between different teeth. Most infundibulae contained quite prominent and extensive lateral branching from the central vascular channel, some of which appeared to be filled with collagenous debris, while others contained some food material, and still others were completely empty. This finding is similar to those of a recent study by Suske et al (2016b) in which the extensive lateral vascular branching surrounding a central vascular channel that varied markedly in size was found on histology in all infundibular cementum. These lateral branches were even too small to be imaged on microCT, although the central vascular channel was usually visible. The resolution of the microCT scanner used in the current study is stated to be 82 micrometres (XTreme CT Specifications, Scanco Medical AG), and so the lateral branches from the central vascular channel of the infundibulum must be less than 82 micrometres in diameter. This corresponds with the findings of a study performed by Kilic et al (1997b), which found the median diameter of cemental vascular channels to be 40.75 micrometres, though separate measurements of lateral branches versus central vascular channels are not specified in that study.

The range of HU measured in abnormal regions of infundibular cementum in this study was wide (2035 HU). The minimum value measured was found to be -692 HU, which is relatively similar to the designated attenuation of air (-1000 HU), while the maximum value was 1343 HU. This value is nearly 1000 HU less than the minimum value obtained for visually normal infundibular cementum on microCT (2029 HU). When measurements of HU were compared with histological findings, the range of attenuation values separated into a group with greater mean attenuation (694 HU) and lesser mean attenuation (-70 HU). These two groups represent those infundibulae filled with cemental fragments along with feed material or those with moth-eaten or severely damaged cementum as compared to those with no filling, respectively. The range in HU obtained for both of these groups individually still remains greater than 1000 HU, indicating that there is great variation in the infundibular contents. Although the detail found in microCT images is not seen by the human eye on a standard CT scan, areas of infundibular cemental defects are readily identifiable as seen in the 100 maxillary cheek teeth examined in this study. In further studies, by measuring HU of areas of cemental defects on both standard CT scan and microCT, these values may be compared to make even a standard CT scan of infundibular cemental lesions more clinically applicable. The microCT values may be transformed to apply to the standard CT attenuation scale to better understand what is contained within any imaged infundibular cemental defect. Currently, the detection of hypoplastic infundibulae on CT scan is usually an incidental finding when the CT examination is performed for other purposes, including when performed due to caries-related clinical disease in another maxillary cheek tooth. However, if CT scans were to be used to assess all infundibulae, it may be possible for the microCT scale to be further studied and extrapolated to standard CT infundibular assessment. This in turn could improve clinical decision-making and allow effective treatment protocols on cheek teeth with infundibular caries.

Overall, the detection of apical cemental hypoplasia or central linear defects on microCT images seemed to have limited bearing on the histological appearance of the infundibular cementum, with extensive lateral vascular branching histologically found even in infundibulae that appeared to have a central defect only on microCT images. On histology, the infundibulae were found to contain areas of total cemental hypoplasia or areas of cementum with varying extents of lateral branching of the central vascular channel. The areas lacking cementum were either totally empty, filled with plant material, or pale pink debris with a collagenous appearance. Infundibulae with occlusal surface caries and some of those that appeared to be unaffected by caries on the occlusal surface both contained feed material deep within the lateral branches of the central vascular channel. Some teeth with obvious occlusal surface caries contained no feed material deep within the infundibulum. Overall, the cemental defects as imaged on microCT did not necessarily closely resemble the cemental structure found on histological examination. In particular, the great variation in histological appearance was often not fully appreciated on microCT. A comparison of the degree of tissue attenuation imaged on microCT with histological findings may allow better understanding of the relationship between radiological imaging and microstructure of the infundibulum, therefore allowing us to more accurately understand the composition of infundibular contents.

5. Conclusions

It is difficult to differentiate between infundibular lesions that will become clinically significant and those that will remain asymptomatic. Even infundibulae that appear grossly normal occlusally may contain subocclusal caries. In this study 14/18 (78%) of control teeth that were completely normal on the occlusal surface were affected subocclusally. Overall, 91% of infundibulae were affected by one or more lesions in this study. In this study, data compiled from visual assessment of intact and sectioned maxillary cheek teeth, standard computed tomography, microCT, and histology were compared to provide a better understanding of the anatomical variation within normal and abnormal infundibulae. Findings on microCT and histological examination showed many infundibular abnormalities to be present, particularly in the cementum. The results of this study confirm the poor relationship between occlusal caries and deeper infundibular cemental defects.

Infundibular lesions were so common in all Triadan positions, age groups, and infundibulae that statistical analysis on prevalence was difficult in this regard. As the cheek teeth were selected for age, significant analysis is not possible on age-related factors, but a relatively similar prevalence of occlusal caries was found in all age groups (59% to 84%). Further research and studies are needed to determine the significance of occlusal surface cemental hypoplasia versus occlusal surface caries in the development of more severe infundibular lesions subocclusally.

Histology revealed many wide channels branching off the central vascular channel which were likely lateral blood vessels that supported the cementum deposition more peripherally but that never filled with cementum during dental development. The occlusal and lateral infundibular vasculature was possibly prematurely occluded in some infundibulae, while larger areas without cemental deposition could also possibly indicate true developmental defects in the cementum. Some areas of abnormal cementum were filled with plant material even deep within the infundibulum, and this food material was likely the cause of most of the discoloured cementum in this study. Even in the infundibulae categorized as normal, there must have been some connection of the subocclusal cemental defects to the occlusal surface that allowed impaction of plant material. Despite the above limitations, the gross pathological and histopathological characterization of normal, carious, and hypoplastic areas of cementum and comparison to their images on standard CT and microCT have allowed a more complete understanding of equine maxillary cheek teeth infundibular pathology.

After further research is performed in this area in the future, it is possible that different guidelines on the diameter of the central vascular channel and appearance of infundibular cementum may be developed to designate normal and abnormal infundibulae. Further studies are also required which observe maxillary cheek teeth infundibulae throughout a horse's life to better characterize the progression and development of infundibular cemental lesions into caries.

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